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EXPERIMENTAL MEDICINE

AN EXPERIMENTAL INVESTIGATION OF THE TREATMENT OF WOUNDS OF THE HEART BY MEANS OF SUTURE OF THE HEART MUSCLE.

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PART I.

INTRODUCTORY.

In attempting to trace the history of the growth of knowledge regarding wounds of the heart, the investigator is soon impressed by the infrequency with which this subject is mentioned by older writers. In the works of Hippocrates and Celsus we find occasional mention of wounds of the heart, always, however, accompanied by the statement that such wounds are followed by immediate death. Paul of Ægina, Roland, Lanfranc give short descriptions of the symptomatology of cardiac wounds, and all agree that death results at once. Toward the close of the fifteenth century this opinion was still prevalent. The beginning of the sixteenth century, however, saw the dawn of a new era. Foreign bodies, most often bullets and arrow-points, had been found healed in the hearts of living animals. Reports were published which claimed that deer, wild boar, and other animals of the chase had, after receiving a wound of the heart, continued to run for minutes and even for hours. Soldiers who had been wounded in the heart were reported to have continued to fight in the excitement of battle for some minutes before death had overtaken them.

At about this period, Ambroise Paré published a report of the now

celebrated case of the Turin gentleman, who, although wounded in the heart during a duel, was able to pursue his antagonist 700 feet before he dropped to the ground and died. The learned societies of those days discussed this case with eagerness and commented on the rapidity of onset of the fatal symptoms after wounds of the heart. As evidence of this one finds occasional short papers on the subject in the medical literature of the seventeenth and eighteenth centuries. The writers of these papers advanced no new theories or new facts, and hence attracted little attention. They regarded these cases of delayed death after cardiac injury either as apocryphal or as mere curiosities of medical literature. It was only in the beginning of the nineteenth century that observers evinced a keener interest in the subject, as is shown by the more comprehensive investigations of Jamain, Sannetti, and others, published at this period, and it remained for G. Fischer to lay the foundation for all future work on this subject by the publication of his exhaustive and critical study of 452 cases of wounds of the heart in man, collected from medical literature.

The main results arrived at by Fischer * may be summarized in brief in the following: Seven to ten per cent of all wounds of the heart heal and the patients recover. In the cases with a fatal outcome, death sometimes occurs simultaneously with the infliction of the injury, more frequently, however, it is delayed a few moments, or less often for hours or days. Wounds of the ventricles occur with much greater frequency than do those of the auricles. Wounds of the right ventricle are more frequent than those of the left (in the ratio of 27 to 22), because in man the right ventricle forms the greater part of the anterior surface of the heart, while the left ventricle is brought forward only during each systole. On the other hand, the death rate after wounds of the auricles is higher than after those of the ventricles, and in this respect the right auricle predominates over the left.

Loison † has recently published a paper, based upon the reports of 277 cases of wounds of the heart in man, which he has collected from the medical literature of the last thirty years. Most of Loison's conclusions agree with those of Fischer. In 39.7 per cent of the patients the right, and in 33.3 per cent the left ventricle was injured. The mortality after wounds of the right was much greater than that after wounds of the left ventricle. In a large proportion of the cases, the fatal outcome was hastened by the over distension of the pericardial sac by the

* G. Fischer, Langenbeck's *Archiv*, 1867, ix, 571.

† Loison, *Revue de Chirurgie*, 1899, Nos. 1, 2, 3, et seq.

blood. Loison declares that the mortality rate varies considerably with the situation, the size and the character of the wound. In general, 85 per cent of all cardiac wounds prove fatal.

It may be said that the gravity of the case depends upon the size of the wound and its location. This does not mean, however, that wounds of small size, *i. e.* punctured wounds, bullet-wounds, are not attended with great danger. Indeed, in many cases these have been shown to cause death sooner than larger wounds. There are a number of factors to be considered which will be referred to later on, but the general statement—the larger the wound of the heart muscle, the more serious the prognosis—will be found to be applicable to most of the cases.

Does death ever occur at the same moment that the heart is wounded? Undoubted cases of this kind have been reported where the whole heart was torn off from the great vessels at its base. If the injury be not so severe as this, however, have we sufficient evidence to warrant the belief that the heart may stop beating at the same moment it is injured? In former times such cases were believed to be of very frequent occurrence. Even Fischer thought he had found reports of several undoubted cases. After having made a careful study of the cases collected by Fischer, I have become convinced that we must exclude most of them from this category, because in the main the reports contain few details and hence are untrustworthy. In only one case, that of the famous soldier L'Atour D'Auvergne,* is the report of the injury and death so clearly told that there can be no doubt of the simultaneous occurrence of the cardiac wound and the soldier's death.

The older writers, as was stated above, believed that death followed quickly after wounds of the heart. They explained this on the assumption of an influence of some kind, reflex or otherwise, upon the nerves or nervous centres of the heart. At the same time, however, they disagreed about the source and character of this innervation, and their successors of to-day have a similar contention. At one time the belief was prevalent that the stimuli for the contraction of the heart passed to the cardiac muscle through the sympathetic ganglia. These ganglia are very numerous in the septum between the two auricles. The experiments on rabbits and dogs by Kronecker and Schmey † showed that there was a spot in the septum between the ventricles of these animals where puncture with a fine needle was followed by immediate stoppage of the heart. They inferred that this was due to some injury to the large nerve-ganglia

* See Fischer, loc. cit. Case 72.

† Kronecker and Schmey, *Sitzungsberichte d. Berliner Akad.*, 1884, p. 87.

in this region and named this spot the "coördination centre." Kronecker and Schmey declared that in all probability a similar spot exists in the human heart. The investigations of Krehl,* Romberg,† W. His,‡ and Schaefer § gave conclusive proof that these nervous ganglia were derived from the sympathetic and that they could, therefore, carry only centripetal or sensory and no motor stimuli. His believes that the heart contains only sensory nerves, while Romberg calls attention to the fact that the fœtal heart contracts rhythmically long before either nerves or nervous ganglia can be found in the cardiac muscle. It is incorrect, therefore, to attempt to explain the cardiac stoppage in the experiments of Kronecker and Schmey, and likewise the possible sudden arrest of the heart after wounds of that organ, as a result of injury to the nerve ganglia in the heart. Engelmann || has shown that it is possible to incise the heart-muscle in such a manner that, while the continuity of the muscle is not totally disturbed, all the nerves are doubtlessly cut across. Nevertheless, muscular contractions continue. Porter¶ concludes that nerve cells, if present, are not essential to long-continued, rhythmical, co-ordinated contractions of the ventricle, and that the causes of rhythmic contractions of the ventricle lie in the ventricle itself.

If there are, therefore, no motor nerves in the heart, and if this organ can contract after all the sensory nerves have been divided, there must be a true automatism of the heart-muscle. This is the prevailing opinion among writers of the present day. Investigators are undecided as to the explanation of the so-called co-ordination centre of Kronecker and Schmey. In man, wounds of the septum ventriculorum, in the region that would correspond to the co-ordination centre in animals, are very rare indeed. It is only on this assumption, however, that we can explain the immediate cardiac arrest and death in the case of the Chevalier L'Atour D'Auvergne. As no other undoubted case of this kind has been reported we must conclude that immediate cardiac asystole and death after wounds of the heart in man, unless indeed the whole organ be torn from its attachment, is excessively rare.

When death is delayed after an injury of the heart, what is the cause of the stoppage of this organ?

It must, of course, be understood that in the following considerations

* Krehl, *Deutsche med. Wochenschr.*, 1889, p. 549.
† Romberg, ibid., 1890, p. 440.
‡ W. His and Romberg, *Archiv f. exp. Path. u. Pharm.*, 1892, xxx, 51.
§ Schaefer, *Verhandl. d. IX Congress. f. innere Medecin*, 1890.
|| Engelmann, *Archiv f. d. ges. Physiologie*, 1896, lxv, 119; 535.
¶ Porter, *Journal of Experimental Medicine*, 1895, i, 319.

no reference will be made to the secondary or complicating causes of death such as sepsis, shock from the severity of the injury and the like, for they have no direct bearing on the subject in hand. There remain for consideration: 1. The effect on the heart of the loss of a large amount of blood; 2, the change in the character of the heart's action as the escaping blood fills and distends the pericardial sac.

That the hæmorrhage from a wound of the heart is usually very great and often sufficient to cause death in a few seconds needs no explanation. Clinical experience has long ago shown that, after the loss of a large quantity of blood, the heart suffers mainly because it has no longer sufficient fluid to "work on." For the blood, circulating through the heart chambers, stimulates the heart-muscle to contract, and when there is little or no fluid in these cavities, the rhythmic contractions and relaxations of the heart-muscle soon cease. The larger the wound and the freer its communication with the surface of the body, the more rapidly will cardiac asystole and death supervene. The same is also true where the heart wound communicates with one or the other pleural cavity into which the heart can pour the blood from the entire body.

If the wound in the heart-muscle, however, permits of the free escape of blood, while the wound of the parietal pericardium is either very small as compared with that in the heart wall, or the pericardial rent becomes closed or partially closed by clots or by the pressure of the soft parts around it, the blood will be unable to escape from the pericardial sac and the latter will thus become more and more distended. The intrapericardial pressure rapidly rises and the pressure exerted upon the heart by the blood in the pericardium becomes very much increased. To this condition Rose,* although he was not the first to call attention to its importance, gave the name of "heart tamponade." Morgagni † had already recognized its danger. Cohnheim ‡ investigated the subject in numerous experiments on dogs. He injected various quantities of fluids into the pericardial sac of the animals and showed that, with the rise of the intrapericardial pressure, the heart's action steadily grows weaker. A point is soon reached where the heart stops beating altogether. Cohnheim demonstrated that the pressure of the fluid in the pericardial sac is mainly expended upon the auricles and the great vessels at the base of the heart. While the ventricles still contract, the auricles and the great vessels are compressed, the entry of blood into

* Rose, *Deutsche Zeitschr. f. Chirurgie*, xx, 329.

† Morgagni, De Sed. et Caus. Morborum, Epist. 69, Sect. 5.

‡ Cohnheim, *Allgem. Pathologie*, i.

the heart grows less and less, the heart pumps itself dry and finally the contractions of the ventricles also cease. In other words, while the causes are different, the results are the same as in death from acute hæmorrhage, *i. e.* the heart ceases to beat because there is no more fluid upon which it can contract. Rose and many writers after him believe that this heart tamponade is in many cases the chief cause of death.

Dyspnœa, cyanosis, small thread-like pulse, the physical signs of distension of the pericardial sac with fluid, together with the characteristic situation of the external wound, the hæmorrhage, and the symptoms of collapse, are the symptoms that are generally described. Some writers have attributed considerable value as a diagnostic sign to a peculiar whirring noise to be heard over the lower part of the heart; but Riedinger,* Rose,† Delorme,‡ Loison,§ consider it as neither constant nor characteristic.

While injuries of the heart are most often followed by death before medical aid can arrive, there are some cases in which the patient survives for a number of hours or days. The treatment of these patients has consisted in general and local measures such as cleansing and packing the external wound, absolute rest, the application of cold to the præcordial region, the administration of morphine and other narcotics, sometimes venesection (Dupuytren, Stromeyer, Rose). If the hæmorrhage continues and the patient grows worse, the case has been considered a hopeless one.

The first step toward a more direct and local treatment was taken by Rose when he recommended that the pericardial sac should be punctured through the fourth left intercostal space, in order to relieve the dangerous symptoms of heart tamponade. If this did not suffice and the dangerous symptoms continued, the pericardial sac should be opened and all clots and blood removed from it. While in some cases the good results of this operation cannot be denied, it seems to me that Rose goes too far when he declares that "the opening of the pericardium and the immediate removal of the heart tamponade deserves to have as important a place in surgical procedure as tracheotomy."

In man, puncture of the pericardial sac and pericardotomy is already a recognized procedure in surgical practice. In the large majority of cases, the pericardial sac can be exposed and opened without injury to the

* Riedinger, Krankheiten des Thorax, *Deutsche Chirurgie*, xlii, p. 180.

† Rose, loc. cit.

‡ Delorme, *Chirurgie de guerre*, 1893.

§ Loison, *Revue de Chirurgie*, 1899, Nos. 1 and 2.

left pleura. But the technique of the operation needs further development, and the anatomical landmarks in normal and abnormal conditions must be more clearly defined. The recent investigations of Voinitch-Sianojensky* and of Delorme and Mignon† constitute a distinct advance in this direction.

It would be manifestly beyond the scope of this paper to describe the various methods proposed for the operations of paracentesis pericardii and of pericardotomy. For these, the reader is referred to the short but comprehensive work of Terrier and Reymond, *Chirurgie du Coeur et du Péricarde.* There are several points, however, to which we would draw attention. Up to within the last few years the belief was widespread that large collections of fluid in the pericardium push the heart backwards against the vertebral column. If this were always true, there would be little danger of wounding the heart during an aspiration unless the aspirating-needle were made to penetrate too deeply. Recent investigations have shown that this belief is not always true. Large effusions into the pericardial sac may and often do push the heart forward against the anterior wall of the chest, in which situation it might easily be injured. The larger the quantity of fluid in the pericardial sac, the more is the heart pushed forward. This has been seen in the human subject by Rehn;‡ experimentally, in the cadaver, by Delorme and Mignon§ and Voinitch-Sianojensky;‖ and in the living animal body by myself.¶ If there are adhesions between the heart and the anterior wall of the pericardial sac, the injury of the heart during paracentesis of the sac may also take place. Kümmel** found adhesions between the heart and the parietal pericardium 2 centimeters above the point where he had aspirated the pericardial sac. Numerous cases have been reported where the heart was actually injured during this procedure. Hence Eiselsberg†† declares, as a result of his experience, that the danger of paracentesis is often greater than that of incision of the pericardial sac. He recommends that in pericardotomy the pericardial sac, when exposed, should be sewn to the edges of the wound in the chest wall,

* Voinitch-Sianojensky, *Archiv. Russ. de Chirurgie*, St. Pêtersbourg, 1897, ii; also *Revue de Chirurgie*, 1898.

† Delorme et Mignon, *Revue de Chirurgie*, 1895, pp. 797; 987, and 1896, p. 56.

‡ Rehn, Langenbeck's *Archiv*, 1897, lv, 315.

§ Delorme and Mignon, loc. cit.

‖ Voinitch-Sianojensky, loc. cit.

¶ See Exps., vi-vii-viii, p. 513.

** Kümmel, see Eiselsberg.

†† Eiselsberg, *Wiener klin. Wochenschr.*, 1895, No. 2.

thus protecting the incision in the soft parts, and the pleural cavities against injury and infection.

While paracentesis or pericardotomy in the treatment of heart tamponade is based on rational grounds, there is at the same time a noteworthy risk connected with its performance. When the pressure on the heart has been removed, the hæmorrhage from the wound in the heart-muscle is apt to begin anew, or, if it had not ceased, to become very much larger. For these reasons, surgeons have often hesitated about following Rose's recommendations, and have sought for other and more efficient methods of stopping the hæmorrhage from a wound of the heart.

Many years ago Billroth declared that no surgeon, who wished to preserve the respect of his colleagues, would ever attempt the suture of a wound of the heart. In 1884 Riedinger* made the oft-quoted statement that "the proposition to suture a wound of the heart, although made in all seriousness, needs hardly a mention." Tillmanns declared in one of the recent editions of his book that surgery is powerless when confronted with a hæmorrhage from the heart-muscle. There were others (König,† Delorme,‡ etc.), however, who dared to give voice to their opinion that suture of a wound of the heart, though difficult and connected with great risks, was theoretically and probably technically possible. On logical grounds one might ask why direct surgical treatment of the wound, according to the general principles of wound treatment, was never proposed and attempted. For had it not been proven centuries ago that cardiac wounds could heal and that foreign substances could heal in the heart-muscle without interfering with its function? Had not the heart been exposed in the operation of pericardotomy and were writers not agreed that this could be done with comparative safety by the use of modern aseptic technique? In answering this question, we must remember that we have to deal with an organ of first importance which is in constant motion, and which, moreover, was believed to be very sensitive to the smallest mechanical insult or injury. It was feared that during the slightest manipulation the heart might suddenly stop, that the mere passage of a needle might be followed by the direst results. Hence cardiac suture was until very recently considered an unwarrantable surgical procedure.

With the growth of such ideas as those of König and of Delorme, investigators turned to the animal heart to gain knowledge and experience on this subject.

* Riedinger, loc. cit.

† König, *Lehrbuch d. Chirurgie*, ii.

‡ Delorme, *Chirurgie de Guerre*, 1893.

Rosenthal,* I believe, was the first to attempt to treat a wound of the heart in an animal by direct means. Early in 1895 he showed to the Medical Society of Berlin a dog whose heart, after resection of the sternum, he had wounded and tamponed with iodoform gauze. The wound healed and the animal survived. Del Vecchio† reported that he was successful in keeping alive a dog that had suffered two perforating wounds of the left ventricle and the subsequent operation of suturing. Salomoni‡ twice wounded and successfully sutured the canine heart. In his cases, after 15 to 20 days, the dogs were killed and the wounds were found healed. About one year later F. Bode§ made a series of experiments on rabbits and published a careful and detailed account of his observations. He made small wounds in the ventricles of the rabbit's heart and then closed these with fine silk sutures. The conclusions he arrived at agree almost entirely with some of Fischer's statements concerning the human heart. In none of Bode's experiments did the heart stop suddenly after the wound had been made nor did the passage of the needle and suture and the tying of the latter ever cause more than a temporary irregularity of the heart's action. He found that wounds of the right ventricle bled more freely and were more difficult to manage than those of the left. Wounds of the auricles were very dangerous and almost always resulted in the death of the animal from hæmorrhage before the suture could be applied. Every time Bode passed a needle and suture through the heart-muscle he observed that the next systolic contraction was somewhat delayed, and that there followed a short period of arhythmical heart's action; nevertheless the heart soon recovered its regular movements. The wounds which he made were very small, however, and the sutures he applied few in number. He states that he is convinced that many of his results can properly be applied to the human heart.

When we turn to the human heart, we find that suture of the cardiac would has thus far been done in nine cases. Some of the reports are very short, and contain few details. Cappelen|| sutured the heart-muscle of a patient with a non-penetrating wound. The patient died 2½ days later from secondary hæmorrhage of the coronary artery. Farina¶ of

* Rosenthal, *Deutsche med. Wochenschr.*, 1895, No. 2.

† Del Vecchio, *Riforma med.*, 1895, ii, No. 79; also *Centralbl. f. Chirurgie*, 1895, p. 574.

‡ Salomoni, *Centralbl. f. Chirurgie*, 1896, No. 51.

§ F. Bode, *Beiträge z. klin. Chirurgie*, 1897, xix, 167.

|| Cappelen, *Deutsche med. Wochenschr.*, 1896, p. 186.

¶ Farina, *Centralbl. f. Chirurgie*, 1896, p. 1224.

Rome closed a penetrating wound of the left ventricular wall by two silk sutures. The patient died of pneumonia one week after the operation and at the post-mortem examination the wound was found firmly healed. At the XXVI Congress of the German Surgical Society held in Berlin, April, 1897, Rehn* reported a successful case of suture of the heart. This occurred in a florist, 22 years of age, who, after being stabbed in the left side of the chest, fell unconscious. After three hours he revived, dragged himself along the ground for about 500 feet and again fainted. Shortly afterwards he was brought to the hospital in a condition of severe shock. In the fourth left intercostal space, three finger-breadths from the parasternal line, there was a stab-wound about one inch long. Cardiac dulness was increased towards the right; dyspnœa was extreme and cyanosis marked. The following morning his general condition was somewhat improved, although he was still suffering from shock. Towards noon of the same day he became very much worse, the dyspnœa and cyanosis had increased, the heart-dulness had extended still further to the right and to the left, and the patient appeared moribund. Rehn having decided to operate made an incision in the fourth left intercostal space and resected part of the fifth rib. The pericardial sac could now be seen. When the left pleura was opened, a large quantity of blood escaped from it. On opening the pericardial sac, a penetrating wound 1½ centimetres long, bleeding freely, was found in the wall of the right ventricle. The edges of the wound were approximated by three sutures. Although the case was complicated by an empyema of the left pleura, the patient recovered and remained well.

Parrozzani† published the report of a patient who received a wound so large that "the little finger could be passed into the ventricular cavity" in the left ventricular wall near the apex of the heart. The wound was closed by two deep and two superficial sutures, and the pericardial sac by six sutures. The wounds healed by primary union and the patient recovered.

In a second case operated on by the same surgeon, a woman received a stab-wound through the left ventricle, which was closed with two silk sutures. The patient died of exhaustion on the second day after operation, but at the necropsy the heart-wound was found firmly closed.

Parlavecchio‡ reports a remarkable case. The patient, a young man,

* Rehn, loc. cit.

† Parrozzani, *Bull. d. r. Acad. Med. di Roma*, 1897-8, xxiii, 243; also *Semaine med.*, 1897, No. 23.

‡ Parlavecchio, *Riforma med.*, 1898.

was able to walk to the hospital, a distance of one-quarter of a mile. He had a wound in the left intercostal space from which blood escaped during each systole of the heart. There was a left pneumo-hæmothorax and heart-dulness very much enlarged; the pulse was weak and irregular and the patient was pale and gasped for breath. Eight hours after admission the heart was exposed and a V-shaped penetrating wound 3.5 centimetres long was found in the wall of the left ventricle. The wound was closed with four interrupted silk sutures. The patient made an uneventful recovery and was discharged cured five weeks after operation.

Ninni* reports the case of a man, 33 years of age, who was stabbed in the chest with a short knife. After walking about 200 steps the patient fell to the ground and was brought to the hospital. He had a wound in the fifth left interspace in the mammary line. No radial pulse could be felt, although the heart was beating tumultuously. The patient remained unconscious and the heart's action became steadily weaker, although energetically stimulated. Without anæsthesia, an incision was made in the fifth interspace from the sternal margin to the mammary line, a second incision parallel to the first in the third interspace, and a third incision connecting these two along the sternal margin. The left pleura was incised and a large quantity of clotted blood allowed to escape from it. The pericardial wound, which was 3 cm. long, was enlarged. In the anterior wall of the left ventricle near the apex was found a wound 25 mm. in length, from which fresh blood was escaping. The wound was closed with two silk sutures and the pericardium by a continuous suture. While the left pleural cavity was being cleared of clots and before the cutaneous flap was adjusted, the patient died.

Pagenstecher† operated upon a young man, 17 years of age, who was stabbed just under the left nipple with a knife. After walking a few steps he fainted and was brought to the hospital, where he soon regained consciousness. Next morning he was operated upon, 6 cm. of the left fifth costal cartilage were resected, the wound in the pericardium was found and enlarged. Three cm. from the apex of the heart there was a wound in the wall of the left ventricle 3½ cm. long. A small stream of bright red blood issued continuously from the wound. After considerable difficulty sutures were introduced and the hæmorrhage was controlled. The patient made a complete recovery.

Suture of a wound of the auricle has been performed, and with relative

* Ninni, *Giornale internaz. d. Sc. med.*, 1899, No. 1.

† Pagenstecher, *Deutsche med. Wochenschr.*, 1899, No. 31.

success, in one case by Giordano.* The external wound was in the third left interspace in the anterior axillary line. After the third and fourth costal cartilages had been resected, a wound two centimetres long was found in the right auricular wall. This was closed by four superficially passed sutures. Nineteen days later, the patient died of empyema, having given no symptoms which pointed to lesions of the heart. At the autopsy the cicatrix of the wound in the auricle could be found only with difficulty.

Thus, of the foregoing nine cases of suture of the human heart, four patients recovered entirely; one case died of a complicating empyema nineteen days after operation, another died of pneumonia one week after operation, a third died from shock before the operation was completed. The other two patients lived for two days and died, the one from a secondary hæmorrhage from a coronary artery, the other from exhaustion.

PART II.

EXPERIMENTAL INVESTIGATIONS.

As a contribution to the development of this branch of surgical procedure, it seemed to me of value to attempt to determine how extensive an injury the mammalian heart could safely withstand, and how extensive a suture could be applied to the heart without interfering with its function. With this purpose in view, the following experiments were made on the heart of the rabbit, although I sometimes, for comparison, examined the heart of the dog. I chose the rabbit for most of the experiments, because in this animal one can expose the heart without opening the left pleural sac. The results obtained in the different animals agreed in all essential particulars.

Under ether anæsthesia, which was employed in all of the experiments, the 3rd, 4th and 5th ribs were exposed by a longitudinal incision through the skin and the costal cartilages cut close to the left border of the sternum. These cartilages could with care be dissected from the muscles around them and reflected outwards. The internal mammary artery was drawn outwards and the triangularis sterni muscle divided near its insertion. In this manner the whole of the anterior surface of the rabbit's pericardium could be exposed. The pericardial sac was then

* Giordano, *Riforma med.*, 1898, Sept. 9 and 10; *Semaine med.*, 1898, p. 407.

sewn to the thoracic muscles around the edges of the wound and then opened by a longitudinal incision. The heart was thus exposed and could be manipulated without danger to the left pleura. In making use of this procedure, I had followed the precepts laid down by Eiselsberg and Gussenbauer in performing pericardotomy in man. I found this procedure very valuable, for I was able thus to avoid almost uniformly the injuries to the pleura which occurred in so many of Bode's experiments, and to which the death of a large number of his animals was attributable.

When the experiments and observations on the heart of the animal had been made and the wound in the wall of the thorax was ready to be closed, the cartilages were returned into place, the cut end of the left pectoralis major muscle sewn to the insertion of that of the right side, the serous surfaces of the outer pericardial layer thus brought together, and by this means the pericardial sac again closed. The skin wound was then closed by interrupted silk sutures and an iodoform collodion dressing applied.

Most of the animals recovered rapidly from the operation. One to two hours after the experiment they were running about as if nothing had been done to them. Every one to two days exact notes were taken as to their condition, character of their hearts' action, etc.

The experiments and the results obtained I have arranged under three headings:

Series I. Wounds were made of various sizes and in various parts of the heart. No suture was applied to the cardiac muscle.

Series II. Suture of the normal intact wall of the heart.

Series III. Suture of the cardiac muscle after wounds had been made in it.

Series I.

Wounds of the heart—their size and situation. The hæmorrhage. The physiological irritability of the heart-muscle.

The heart was exposed in the manner above described and manipulated in various ways. The organ could be lifted well up into the thoracic wound without causing any appreciable change in the heart-beat. It could even be drawn forward so far that the greater part of the ventricles lay outside of the thorax, so that all sides of the ventricles could be thoroughly examined. When part of the heart-

muscle was grasped and gently compressed with a forceps, no appreciable difference in the character of the heart's action was observable. When, however, firmer pressure was made, a peculiar and characteristic irregularity was noticed. This irregularity was also to be seen when the heart-muscle was punctured with a coarse teasing needle. Every time the needle penetrated the heart-wall, there was a delay in the occurrence of the next systolic contraction, followed by a short period of very irregular action of the heart. This irregularity was usually noticeable during the time the needle was penetrating the epicardium and the moment that the endocardium was pierced. After a few moments the heart's action becomes regular again.

Several times, in order to determine just when the arhythmia

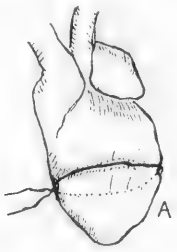

FIG. 1.

Transverse Ligation of Ventricles.

occurred, I proceeded in the following manner. I had noticed in previous experiments on the rabbit's heart that it was possible to tie a ligature transversely around a portion of the ventricles without more than temporarily disturbing the heart's action. (See Fig. 1.) Though the ligature was drawn very tightly, the part (A) below the ligature continued to beat, though somewhat more weakly than the rest of the ventricles.

When the ligature was tied, there was only a temporary irregularity of the heart lasting from two to five minutes. If anything less than one-half of the ventricles was thus tied off, the heart's action remained strong and regular. When the ligature was removed, the heart's action became very rapid, there being often 300 beats to the minute. In less than five minutes, however, the beats became slower

and soon returned to the normal. If more than one-half of the ventricles was tied off in this manner, the action of the heart at once became weak and very irregular and, unless the ligature was at once removed, stopped in less than one minute.

I tied off a part of the ventricles in this manner, and made a large incision penetrating into one ventricular cavity. On account of the ligature around the ventricles above the line of incision, there was no hæmorrhage. When the heart's action had returned to the normal, a needle was pushed through the heart-wall near the line of the incision. It was forced forward very slowly, so that the heart-muscle was penetrated layer by layer. The exact moment when this needle penetrated the epicardial layer and, likewise, when it passed through the endocardium, could be determined. While the needle passed through the epicardium the cardiac action was very irregular. Although at first I thought that the heart sometimes omitted a beat, I soon observed that the irregularity was due to a delay in each systolic contraction of the ventricles. With the needle in this position, and supposing that the irregularity would persist, I waited, but found that it disappeared in less than one minute. When I then pushed the needle slowly forward through the myocardium, the heart continued to beat regularly until the needle reached and passed through the endocardium. Then each systole again "lagged behind." Although the needle was left in place, passing through the entire heart-wall, the action of the heart became regular in less than one minute and remained so. I consider it noteworthy that the heart did not omit a single beat during the whole of this experiment.

This irregularity occurs also when the auricles are similarly punctured. As the walls of the auricles are very thin, their different layers cannot be injured separately, and, therefore, the exact moment when the arhythmia occurs cannot be determined. I was much surprised to find in two of my animals, however, that this irritability of the epicardium and endocardium was altogether wanting. Bode believed that such cardiac arhythmia occurred only during injury of the endocardium. My observations seem to show that both epicardium and endocardium react in this characteristic manner.

Heitler,* in his investigations on the electrical excitability of the heart-muscle, arrived at results which substantiate mine. He found that the passage of a weak galvanic current through the epicardial and the endocardial layers of the heart-wall caused an irregularity of the heart's action, while the myocardium did not react to the electric current.

The hæmorrhage that occurs from needle punctures of the heart in the animals experimented on is always small when the ventricles are injured, but more considerable when the needle is made to penetrate the auricular wall. In the latter case, however, as well as in punctured wounds of the ventricles the hæmorrhage soon ceases. Even when the needle is passed through the whole thickness of the heart, thus traversing the walls of both ventricles and the interventricular septum, the hæmorrhage was never so great as to endanger the animal's life. If the animal be killed 3 or 4 days after the experiment and the heart be then examined, the point of puncture can scarcely be seen as a slight depression on the surface of the heart; after one week it is impossible with the naked eye to find the point.

In one animal the heart was punctured in various directions a number of times. The hæmorrhage from the wounds was controlled by compression with gauze and the animal recovered.

The range of experimentation was enlarged by incisions in the cardiac muscle in various directions made by a scalpel with a blade one millimetre in breadth. It is a fact, so well known that it needs no comment, that the heart of the rabbit can be made to beat very slowly by allowing the animal to inhale a few drops of chloroform. By means of this expedient, it was possible for me to inflict the wounds with my scalpel during the systole or the diastole of the heart at will. Wounds that are made during the systole of the injured part bleed more than those made during the diastole. For a wound that is made during systole becomes larger with the diastolic relaxation of the heart-muscle, while, vice-versa, a wound that is made during the diastole becomes smaller with each systole. Thus a wound of a ventricle 2 mm. long, made in systole, would be almost 3 mm.

* Heitler, *Wiener klin. Wochenschr.*, 1898, Nos. 3 and 8.

in size during the diastole; and one of 2 mm. made in the ventricular wall during a diastolic relaxation, would become almost as small as one millimeter during the systolic contraction.

The hæmorrhage from wounds that penetrate into one of the heart-cavities is larger than that from non-penetrating wounds. Wounds penetrating perpendicularly bleed more than those passing obliquely through the heart-wall. In the latter case, the wound canal is longer and, therefore, a condition more favorable to the coagulation of blood in it exists. Also in an oblique wound canal the wound surfaces are more tightly pressed against each other during the systolic contractions of the part. (See Fig. 2.)

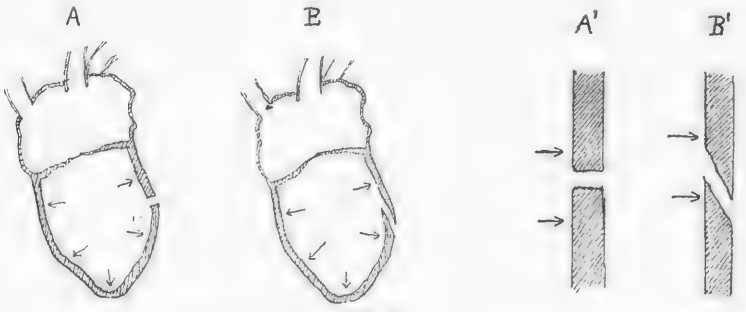

FIG. 2.

To illustrate the Closure of a Wound passing obliquely through the Heart-Wall. (The Arrows represent Lines of Force.)

Wounds of the right ventricle bleed more than do those of equal size made on the left side. Although the pressure of blood in the left ventricle is far greater than in the right one, and, therefore, the tendency to washing away of the clots in the wounds far greater, nevertheless, wounds on the left side are closed by coagulation more rapidly than on the right side. This will be readily understood if we take into account the greater thickness of the wall of the left ventricle and, hence, the greater length of the wound canal. In addition to this, the blood in the right ventricular cavity contains a larger amount of CO_2 and, hence, coagulates more slowly than that in the left. Thus a penetrating wound 2 mm. long could be made

in any part of the left ventricle and the hæmorrhage could be stopped by means of a gauze compress. Such wounds will often close without suture. If the wound in the left ventricle is larger than 2 mm., the animal regularly bleeds to death unless a suture is applied. Wounds 2 to 3 mm. long in the right ventricle of the rabbit will regularly cause fatal hæmorrhage unless the wound is sutured. If the suture be not employed in even the smallest incised wounds made in the auricles, the animal will bleed to death.

The hæmorrhage from all these wounds was most frequently systolic; during each contraction a small stream of blood squirting from the wound. Diastolic bleeding was, however, occasionally observed, especially where the wounds were very large. If the wound is very large (4 or more mm. in the rabbit; 6 to 20 mm. in the dog) and gapes, the blood will simply run out of the wound during the diastole. The same can occur where the opening is of such a size and shape that it closes during each systole and gapes during the diastole of the part. The systolic hæmorrhage is always one under pressure and, hence, greater than the diastolic bleeding.

Series II.

The number of sutures that may be applied to the heart-muscle without interfering with its functional activity. The method to be followed.

The purpose of this series of experiments was to determine the amount of suturing that the heart could stand and the best method of passing the sutures.

In sewing the heart-muscle, the stitches may be passed through a part or through the whole thickness of the cardiac wall. There are some advantages in having the suture pass through a small part of the muscle. In the first place, although the hæmorrhage from the needle puncture is never considerable, except when a blood-vessel is injured, its quantity is proportional to the amount of heart-wall punctured by the needle. When the suture passes through the pericardial layer only there is generally no bleeding at all. In medium-

sized dogs, the visceral pericardium measures about one-half a millimetre in thickness. Unless a large vessel be injured, the passage of a suture through this layer occasions no bleeding at all. The deeper the suture is passed, the larger is the hæmorrhage.

In the second place, the pericardium, being the toughest part of the heart-wall, is less apt to tear when little muscular tissue has been included in the suture with it. Should the suture tear through the muscular layer, if a considerable part of the latter is included, it is also very apt to tear out through the pericardium. This accident happened in several of my experiments.

As the size of the animal increases, so also does the force of each contraction of the heart become greater, and hence the greater is the strain that the heart-muscle and the sutures have to withstand. In the dog's heart the sutures that included a considerable part of the thickness of the wall tore out oftener than did those similarly passed through the cardiac muscle of rabbits. In other words the larger the animal, the more apt is a deeply passed suture to tear out. I believe, therefore, that sutures which penetrate through the epicardium and superficial layers of the myocardium only are most certain to hold. The sutures can be passed to this depth with a minimum amount of bleeding and a maximum of certainty that they will hold. Where, however, a very large wound exists and this superficial suture would not suffice to approximate the wound surfaces, a deeper suture or two tiers of suture must be applied.

Another point of technique to which I must call attention is that the sutures should be drawn tight and tied during the diastole of the part operated upon. In the heart of the smaller rabbits this was often impossible on account of the great rapidity of the heart's action. In the larger rabbits and in the dogs, however, where the heart does not beat so rapidly, it is always possible to tie the sutures during a diastolic relaxation. The strain upon both the suture and the heart-muscle is less during both systole and diastole when the suture is tied during diastole than when it is tied during systole. The results I obtained in my experiments with this "diastolic suture" have been very satisfactory. And I may here state that, from the time that I

passed my sutures only through the superficial layers of the heart-wall and tied them only during a diastolic relaxation of the part operated on, I saw very few of my sutures tear through the muscle and so become loose. Suture of the ventricles is easier than that of the auricles, because the former are easier of access and their walls thicker.

In order to discover the maximum amount of suture that the heart could stand, I made a number of interrupted and continuous sutures extending over various distances of the heart-wall. Regarding the advantages of the interrupted over the continuous suture, I shall speak in Series III. The functional activity of the rabbit's heart is not interfered with even by a very extensive line of stitches. In several animals (see Experiments XXIV and XXXIII, pp. 516 and 518), a continuous suture was applied beginning at the base of the right ventricle and extending to the apex and continuing over the wall of the left ventricle to the left auriculo-ventricular sulcus. All the animals operated on in this way recovered and remained well during the full time of observation (3 to 12 weeks). In one animal the suture ran over a part of the auricles also (see Experiment XLI, p. 518). This animal likewise remained well. Of the histological changes in the hearts of these animals I shall speak later. It may be said in this place, however, that the pathological changes consisted mainly in a connective tissue proliferation between and for a small distance around the sutures, while the adjoining muscular fibres preserved their normal appearance.

Series III.

Large wounds of the heart in the rabbit and their suture.

In this series of investigations, I obtained most unexpected results. Small wounds (2 to 5 mm.) of the rabbit's heart when sutured, heal rapidly and well, so that the process of cicatrization of the wound is completed in about 14 days. The interrupted suture has many advantages over the continuous suture. The former, it is true, takes a somewhat longer time in its application than the latter, but it is more certain to hold. If one stitch of a continuous suture should tear out, the whole suture would naturally become loosened, with the most

disastrous results. Besides, the interrupted suture does not compress so many bundles of muscle fibres as does the continuous (see Figs. 3 and 4). My histological investigations have shown me that this is a distinct advantage, because all the muscle fibres that are compressed

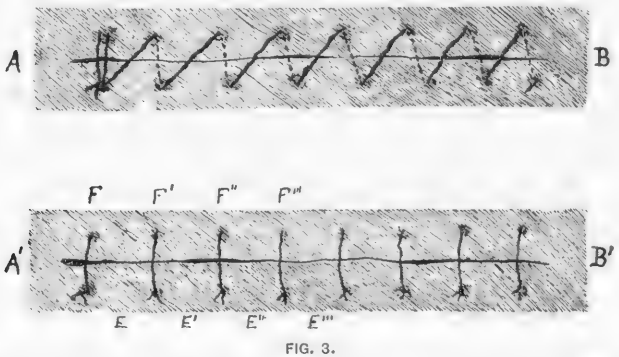

FIG. 3.

The Continuous and the Interrupted Suture. Compare this with Fig. 4.

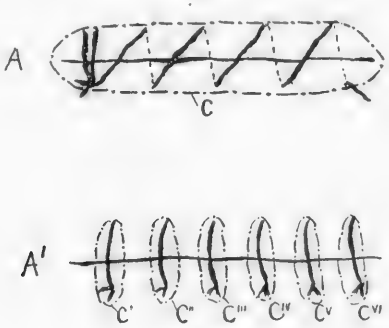

FIG. 4.

The broken line C in A is to show the area within which the muscle fibres are compressed by the continuous suture AB (Fig. 3). A′ represents the condition in the case of the interrupted suture A′ B′ (Fig. 3).

by the ligature or suture eventually atrophy and are replaced by connective tissue.

In very large wounds (4 to 10 mm.) of the heart in rabbits, the hæmorrhage is of course profuse, and unless some method to stop the

bleeding is used and the suture rapidly applied, the animal will bleed to death in a few moments.

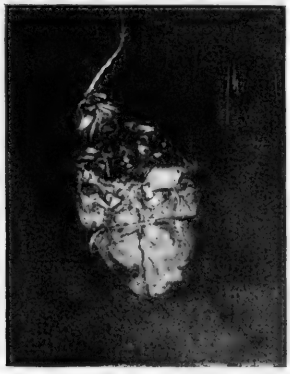

FIG. 5.

To illustrate the Size of the Incision made in Exp. XXXVIII. Compare with this Figs. 6 and 7.

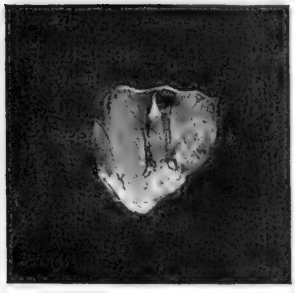

FIG. 6.

Cut Surface of Heart Two Months after Exp. XXXVIII. (Light part of figure is the scar tissue.)

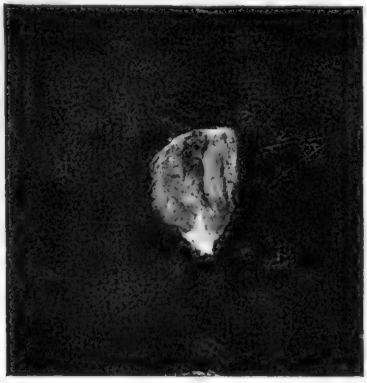

FIG. 7.

Section of Heart of Rabbit (Exp. XXXVIII). Incision is at right angle to that in last figure.

I have described, in Series I, the method by means of which a ligature may be tied around a part of the ventricles (p. 492, and Fig. 1). It occurred to me that by means of this expedient, I should be able

to make very large wounds in the ventricles of my animals by a true "bloodless method." Thus in a number of experiments, after the temporary ligature had been applied, a small scalpel was pushed through the whole thickness of the heart, perforating first the left ventricular wall, then the septum, then the right ventricular wall, and then made to cut downward through the heart-muscle until the

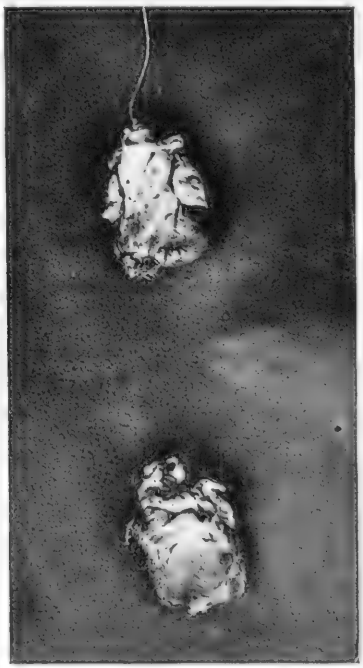

FIG. 8.

Rabbit's Heart, Three Months after the Removal of the Lower Third of the Ventricles. (Experiment XXI.)

knife emerged at the apex of the heart. In this manner, the lower part of the heart was cut into two parts and both ventricular cavities opened. On account of the provisional ligature applied above the highest point of incision there was no hæmorrhage. The whole wound about 2 cm. long, was closed by interrupted sutures of fine silk. The provisional ligature was then removed. Of the 6 ani-

mals thus operated on, one died of hæmorrhage because, during the manipulations, the ligature slipped, a second animal died from sepsis, the remaining four recovered and remained well during the full period of observation (1 to 12 weeks). (Experiments XXVI, XXVII, XXXI, XXXVIII, XXXIX, XLII; see Figs. 5, 6 and 7.)

In a similar manner, the incision was made transversely to the axis of the heart extending over the whole wall of one ventricle. This large gaping wound healed and the animal remained well.

In another series of animals, a tobacco-pouch suture was applied around the heart, so that one-fifth, one-fourth, one-third and even almost one-half of the ventricles was below the ligature. This was then drawn fast and knotted. The part of the heart below the ligature was then cut off and the raw surface covered over by a sero-serosa suture. A number of animals thus operated on remained well (Fig. 8).

In like manner one or both auricular appendages and, with them, parts of the auricles themselves could be tied off and cut away.

And, finally, it must be mentioned that in not a single one of all my experiments did sudden cardiac arrest occur.

PART III.

THE HISTOLOGICAL CHANGES THAT OCCUR IN THE PROCESS OF HEALING OF CARDIAC WOUNDS.

While the literature concerning the histological changes that take place in the healing of wounds of the voluntary muscles is large, that dealing with the healing of wounds of the heart is very small.

Histologically, the cardiac muscle is peculiar in that its fibres contain transverse striations like all voluntary muscles, but that they are not controlled by the will. The muscular fibrillæ have no sarcolemma and the fibres anastomose with each other, so that the "whole heart may be regarded as a single branching muscle fibre, whose branches anastomose with each other."

It seems to be fairly certain that in the healing of wounds of the voluntary muscles, a regeneration of the muscle fibres is to some extent possible. A large number of these young muscle fibres

eventually atrophy and are replaced by connective tissue, but some of them remain as regular muscle fibres. Authorities differ as to the origin of these new fibres. While Waldeyer,* Weber,† Hofmann,‡ Robert,§ Zenker‖ and others declare that the new fibres are derived from the spindle cells in the interfibrillar connective tissue, other investigators (Renaud,¶ Virchow, Duplaix,** etc.) believe that the new fibres are derived from the old ones.

The question whether a regeneration of the muscle fibres of the heart ever takes place, is one concerning which there is also great difference of opinion. Heschl†† believes that he has frequently seen new muscle fibres in microscopic specimens from hypertrophic human hearts. Goldenberg‡‡ declares that it is possible that in hypertrophy of the heart "new muscle fibres are formed by a longitudinal division of the old fibres." Tangl,§§ on the contrary, denies that a regeneration of the heart-muscle fibres occurs. He never found cells which he would acknowledge to be transitional forms between a connective-tissue cell and a muscle-cell, and never saw an undoubted mitotic division of the nucleus of a muscle fibre.

Bonome‖‖ and Berent¶¶ investigated this subject experimentally on the rabbit's heart; their conclusions on this point agree with those of Tangl.

The method in my examinations was the following: Each animal was killed by a sharp blow on the back of its neck, the heart was quickly removed and opened, and the tissues fixed by immersion for 24 hours in a 10 per cent solution of formalin in water or in Müller's fluid. The specimens were hardened in alcohol of increasing con-

* Waldeyer, Virchow's *Archiv*, xxxiv, 473.
† Weber, ibid., xxxix, 210.
‡ Hofmann, ibid., cl, 161.
§ Robert, Ziegler's *Beitrage*, x, 109.
‖ Zenker, *Verhandl. d. X. Internat. Congress.*, 1890, ii.
¶ Renaud, *Gaz. méd. de Paris*, 1877, p. 361.
** Duplaix, *Archives de med. gén.*, 1885.
†† Heschl, *Comp. d. path. Anat.*, p. 176.
‡‡ Goldenberg, Virchow's *Archiv*, ciii, 88.
§§ Tangl, ibid., cxvi, 432.
‖‖ Bonome, Ziegler's *Beitrage*, 1889, v, 267.
¶¶ Berent, Inaug.-Dissert., 1887.

centration, embedded in celloïdin, sections cut, and then stained with hæmatoxylin (Delafield), Van Gieson's fluid, Bismarck brown, carmine, and Weigert's fibrin stain.

In the normal heart-muscle of the rabbit the longitudinal is much more marked than the transverse striation. The nuclei are long with rounded ends and generally stain strongly with the ordinary staining dyes.

Within the first 24 hours the whole wound area is infiltrated with red and white blood cells; there is fibrin between the edges of the

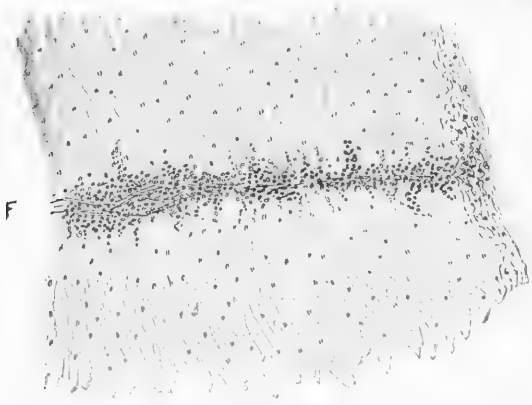

FIG. 9.

From Wound of Rabbit's Heart 24 hours after operation. Fibrin between the lips of the wound; beginning round-cell infiltration. X60.

wound and between the neighboring muscle fibres (see Fig. 9). The interfibrillar capillaries are distended with blood. In a few instances a well-marked "fragmentation" of the muscle fibres could be seen.

After 48 hours the wound area is so thickly infiltrated with leucocytes that it is often difficult to find the exact location of the wound. The fibres that are compressed by the sutures are undergoing degeneration, as is shown by their granular aspect and by the fact that their nuclei will often not take any stain. Around the sutures the infiltration of small round cells is very large. The nuclei of the muscle cells near the wound seem to be increased in number and

some seem to be undergoing amitotic division. In the degenerated muscle fibres, the protoplasm is broken up and collected in little masses. Among these fibres I have occasionally seen nuclei undergoing karyokinetic changes. I have never, however, been able to feel convinced that these nuclei did not belong to the interfibrillar stroma, or, perhaps, to the capillary wall and not to the muscle fibre.

By the fourth day the area of operation is still thickly infiltrated with small round cells. The degenerated muscle fibres have begun to disappear. Numerous young spindle cells can now be found. Their shape and size vary considerably, and they are very frequently

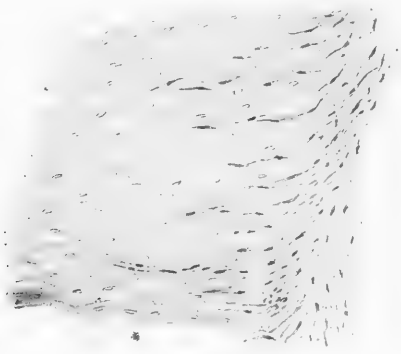

FIG. 10.

To illustrate the Growth of the young Spindle Cells between the muscle fibres. From the heart of the rabbit eight days after operation. X60.

encountered in and just under the pericardium and around the sutures. The nuclei in some of these cells are undergoing mitotic division. Around the sutures just under the pericardium are to be found some large giant cells with numerous nuclei.

On the 6th or 7th day the whole area of the wound is filled by these spindle cells; they have replaced all the degenerated muscle fibres and have even penetrated in places between the uninjured ones.

By the 10th day the granulation tissue is beginning to change into fibrous tissue, the spindle cells grow longer and thinner, and the small round cells are disappearing (Fig. 10). Giant cells still occur

in the neighborhood of the sutures. This new-formed connective tissue is much larger in amount under the pericardium than near the endocardium, so that one has the impression of its being derived for the most part from the subepicardial connective tissue.

At the expiration of four weeks, the whole wound is filled by the connective tissue, which extends for various distances into and between normal muscle fibres. This connective tissue is especially prominent, as stated above, between and around the sutures. In the heart where,

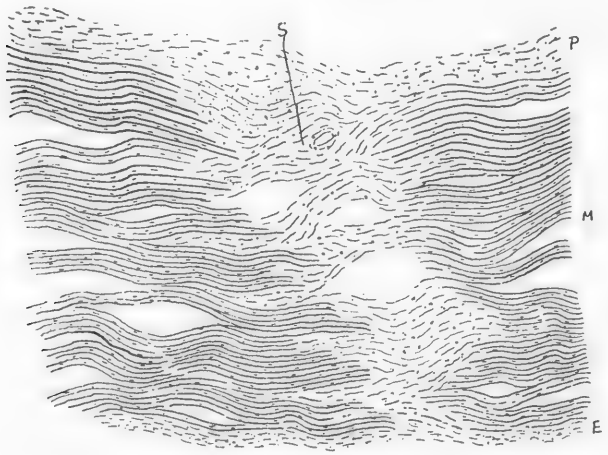

FIG. 11.

Cross-Section of perforating Wound of Rabbit's Heart three weeks after operation. P, Epicardium; M, Myocardium; E, Endocardium; F, Fibrous Tissue of Scar. (Semi-diagrammatic.) X40.

without previous injury to the organ, a continuous suture has been passed, the whole area of the line of sutures is transformed into connective tissue, so that there is a band of fibrous tissue running along the heart-wall (Fig. 11).

In the hearts in which an interrupted suture has been made, the condition is entirely different. In and around each suture there is considerable connective tissue formation, but between each of two adjacent sutures, some normal muscle fibres remain. Thus in Fig. 3

transverse section through any part of the line of suture AB would show connective tissue bands, while transverse section through any of spaces, E, E′, E″, E‴, etc. (Fig. 3, A′B′), would show that in this situation the muscle fibres have been preserved. Near and around the sutures, F, F′, F″, etc., the connective tissue would have replaced the muscle tissue.

CONCLUSIONS.

It would, of course, be incorrect to attempt to draw conclusions as to the dangers and the chances of success of suture of cardiac wounds in man from the results obtained by animal experimentation. Animals are placed in very unfavorable conditions after the operation. They are very restless and cannot be kept quiet. Ideal cleanliness is impossible and the animals may infect their wound by rubbing the external wound against the dirt on the floor of their cage. From the animal mortality in these investigations no rigid inferences applicable to human beings can therefore be made.

Some conclusions of importance can, however, be drawn. Above all, my experiments seem to show that the mammalian heart will bear a much greater amount of manipulation than has hitherto been suspected. Very large wounds of the heart can heal and the healing process occurs in a manner entirely analogous to that in other muscular tissues. Even an extensive suture of the heart-wall of rabbits and dogs, although we know that thereby a large number of muscle fibres are destroyed and replaced by connective tissue, does not interfere with the function of the cardiac muscle as a whole.

Can some of the results in the above recorded experiments be, with some restrictions of course, applied to the human heart? I think that this question must be answered in the affirmative. If we compare the knowledge we possess of wounds of the heart in man, with that obtained from animal experiments, and find that they agree in all essential particulars, then we are justified in reasoning by analogy that suture of wounds of the heart in man will give results similar to those obtained in the animal. In the last few decades, the advances made in all the branches of medicine—especially in pathology, bacteriology

and surgery—have been due to a great extent to the generalization of the results of animal experimentation. To the careful and critical investigator, the results obtained in the animal experiment have always been of the greatest value in indicating to him the possibility of results to be obtained by similar procedures in the human body.

From the study of wounds of the heart in man, and from the results obtained in my experiments, this conclusion seems therefore justified: wounds of the heart in man, when all other means have been tried and found wanting, can and ought to be closed by suture. The application itself of the suture is devoid of the one great danger that was feared in the past, *i. e.* of sudden arrest of the heart during the manipulations incident upon the application of the sutures. The number of sutures should be as small as possible so as to limit the amount of connective tissue which will be formed; for all the muscle fibres that are compressed by the sutures eventually atrophy and are replaced by new-formed connective tissue. It is probable that this connective tissue will not lead to degenerative changes in the heart-muscle. On the post-mortem table, fibrous plaques are often found in the otherwise normal human heart. In a number of the muscles of the body fibrous bands—tendinous intersections as they are called—are normally found. In the large number of microscopic sections of the heart-muscle that I have examined, I could find no evidence of pathological changes in the muscle fibres some distance from the scar. For similar reasons the suture should always be an interrupted one. We have shown that there are dangers and disadvantages in the continuous suture both on theoretical grounds and in practical use.

The sutures should be passed through as little of the heart substance as possible; if they penetrate the epicardium and a small part of the thickness of the heart-muscle it will generally be sufficient.

When the heart's action is not too rapid, each suture should be tied during a diastolic relaxation of the part under treatment. On this point we have not yet any experience in man. Cappelen,* in his patient, tied the sutures during systole. Rehn† tied them in his case during diastole. Only time and further experience will show

* Cappelen, loc. cit.
† Rehn, loc. cit.

how much importance is to be attached to this point. All that can be said, in the present state of our knowledge, is, that on theoretical grounds and from animal experimentation, it must be considered safest to tie the sutures during diastole.

On first sight, it might appear difficult to apply sutures to an organ in such constant motion as is the heart. In practice, however, the difficulties have been proven not to be so great as might appear.

The heart may be grasped with a forceps and the needle and suture easily passed. It is no more difficult to pass and tie a suture in a large dog than in a small rabbit. Hence we should infer that the difficulties of this procedure in the human heart, are not so great, a fact that has been borne out by the experience of those surgeons who have reported cases of heart-suture in man.

The cases will always be few in which this extreme method of treatment—for so we must style it—is necessary. Indeed, of the patients that come under the care of the surgeon, there are some who will recover from even large heart wounds without any local treatment at all. Cases have been recently reported by Conner, Brugnoli, Hamilton and others, where after wounds as large as three centimetres, the hæmorrhage ceased spontaneously and the patients recovered. One cannot say, therefore, that wounds larger than a certain size must always be sutured. Each case must be carefully considered by itself.

When we examine the nine cases of suture of the human heart in man (see pages 487 to 490) we cannot but hope for considerable success from this new method of surgical procedure. Of the nine cases, four recovered entirely, and four died of complications referable to other organs—quite an encouraging record in a few cases.

Finally, I may be permitted to summarize these conclusions as follows:

1. Suture of a wound of the heart as a final resort is an operation worthy of consideration in some cases and often justifiable.

2. Suture of wounds of the heart in animals, and also in man, is devoid of the danger of sudden arrest of the heart, due to the manipulation of the heart incident to the procedure, unless Kronecker's coördination centre be injured.

3. The suture should be an interrupted one of silk, applied in most cases so that the epicardium and superficial layers of the myocardium should be the only ones penetrated, and tied, when possible, during diastole.

4. No stated indications can be given as to the cases that are operable or the time when the operation should be done. Each case must be considered by itself for symptoms which would justify operative interference.

In conclusion, I desire to take this occasion to state that through the kindness of Dr. J. Mikulicz, Professor of Surgery in the University of Breslau, the laboratory of the Surgical Clinic was placed at my disposal, and that the greater number of my experiments were made in that laboratory.

PROTOCOLS OF EXPERIMENTS.

I. 14-vi-'98. Rabbit. Weight 1480 gm.; resp. 132; pulse 240. Ether narcosis. Median incision 4 cm. long. Pectoralis major cut near its insertion and reflected outwards. Cartilages of 4th and 5th ribs cut close to the left border of the sternum and also turned back, together with the muscles attached to them. Triangularis sterni cut. Internal mammary artery drawn outwards. Pleura intact. Bleeding small. Pericardium exposed on its whole anterior surface. Heart's action regular. It is uninfluenced by pressure on the heart with a blunt instrument through the pericardial sac. Pericardial sac now sewn to the thoracic muscles around the edges of the wound, and then opened by a longitudinal incision. Heart's action still regular. Light pressure on heart with blunt forceps has no appreciable influence on heart's action. When firmer pressure is made, the heart lags behind for a moment, and then beats very rapidly. The action of the heart seems to be unaffected by pressure on the auricles. With a small teasing needle the left ventricular wall is punctured. The next systole is now delayed for a moment, then follows a short period of irregular contractions. The needle is then made to penetrate the cardiac muscle deeper and deeper. As the needle penetrates the endocardium the same arhythmia is observed. The needle is now withdrawn. There is some hæmorrhage for two minutes. The heart is now punctured several times in several parts of the right and left ventricles, the needle penetrating each time into the cavity of the heart. The bleeding from all these punctures is small and soon ceases. Toilette of the pericardial sac. Ribs bent back into place. The cut edge of the left pectoralis major is now sewn to the insertion of the right pectoral muscle. Skin closed by continuous silk suture. Iodoform collodium dressing. After the operation animal is weak on its front legs, probably due to the manner in which it was tied down. Heart's action, as heard with stethoscope, weak but regular. Loud pericardial friction sound. R. 120; pulse 260.

After one-half hour animal is running around and eats grass.

15-vi-'98. Animal seems to feel well; it runs around as if nothing had been done.

17-vi-'98. Pericardial friction sound has disappeared. Heart's action regular, 200 to the minute. Resp. 68.

19-vi-'98. Skin wound healed. Animal well.

26-vi-'98. Animal seems to be well in every respect.

27-vi-'98. Killed. Post-mortem examination: Skin wound clean and healed; pericardial sac closed, adherent to heart over its greater part; small abscess under fifth left rib. On the surface of the heart, the points of puncture can be seen as small depressed spots.

II. 15-vi-'98. Rabbit. Weight 1550 gm.; pulse 180; resp. 80. Ether narcosis.

Incision as in Experiment I. 3rd, 4th and 5th cartilages cut close to left border of sternum and reflected. Internal mammary artery was torn but caught and tied without much loss of blood. Pericardial sac sewn to muscles in usual manner and opened as in last experiment. Heart is now firmly grasped with forceps and pulled out of the wound. Delay in next systole, followed by irregular heart's action for one minute; then regular but rapid contractions. Animal is now made to inhale three drops of chloroform. Heart's action becomes very slow, about 60 to the minute, then more rapid as the chloroform is taken away. With a small scalpel, the left ventricular wall is punctured during its diastole (inhalation of chloroform). Immediately there is considerable hæmorrhage, the blood squirting about 30 to 40 centimetres high at each systole. There is no diastolic loss of blood. While the scalpel penetrated the pericardium and likewise the endocardium, marked irregularity of heart's action. Wound tamponed with gauze; after about three minutes, tampon removed and hæmorrhage has ceased. Animal is again allowed to inhale a few drops of chloroform. Heart's action at once becomes very slow and after a few beats ceases altogether. Chloroform is at once removed. Animal cries out and has a general convulsion. Heart can be seen to be filled by dark blood. Heart again begins to beat, slowly at first, then more rapidly, and at same time wound begins to bleed again. Hæmorrhage again ceases after tamponade. Thorax wound then closed in the usual manner. Duration of operation 38 minutes. After two hours, R. 60; P. 200. Animal is eating grass.

17-vi-'98. Animal appears to be well. Skin wound firmly closed.

19-vi-'98. R. 68.

26-vi-'98. Animal perfectly well.

27-vi-'98. Killed by blow on back of neck. Post-mortem examination shows that pericardial sac is closed and adherent to thoracic wall in front. Parietal pericardium adherent to the visceral layer only at point of wound in left ventricular wall, where both seem much thickened. Pleura intact. Cardiac wound firmly closed.

III. 15-vi-'98. Rabbit. 1400 gm.; R. 68; P. 260. Ether. Operation in every respect like Experiment II, except that wound in left ventricle is made during the systole of the part. Hæmorrhage is much larger than in preceding experiment. It is systolic in character but there seems to be also

some slight diastolic oozing of blood. Same irregularity of heart's action as in previous experiments. Wound, which is 3 mm. long, in transverse axis of heart, does not gape. Pressure applied to it by means of tampon of gauze. Hæmorrhage ceases but begins anew as soon as the gauze is removed. Bleeding is very large; heart's action becomes slower and irregular, hæmorrhage less; heart finally stops in diastole. Post-mortem examination: Wound 4 mm. long in left ventricle, heart chambers almost empty, marked anæmia, of all the abdominal organs.

IV. 15-vi-'98. Rabbit. 1550 gm.; R. 68; P. 285. Ether. Usual incision; 4th, 5th and 6th cartilages reflected. Left pleural cavity opened, but at once closed with gauze tampon. Pericardial sac sewn to muscles of thorax and opened. During the inhalation of a few drops of chloroform, an incision of 3 to 4 mm. in length is made in the wall of the left ventricle during a systole. Strong systolic bleeding. Heart is at once drawn forwards and wound closed by means of pressure with anatomical forceps. Five interrupted sutures applied. These penetrate only the pericardium and superficial layers of myocardium. Bleeding from needle punctures is small and ceases as soon as suture is tied. Heart's action irregular while wound is made and sutured. Thorax wound then closed in the usual manner. It is to be noted that the compression of the edges of the wound in the heart with the forceps, had no appreciable influence on the character of the heart's action.

17-vi-'98. Animal eats and seems well.

19-vi-'98. Skin wound healed.

29-vi'-98. Wound healed. Killed in usual manner.

Post-mortem examination: Parietal pericardium adherent to visceral layer over large part of surface of heart and considerably thickened over situation of wound in heart muscle. Wound in ventricular wall firmly cicatrized and the scar seems somewhat depressed.

V. 17-vi-'98. Rabbit. 1940 gm.; R. 60; P. 280. Ether. Pericardial sac exposed and opened in the usual manner. During a diastole of the ventricle, incision 3 mm. long in middle of left wall, and not perforating into heart cavity. Heart's action remains regular. Hæmorrhage is large and systolic. Bleeding wound clamped by means of artery forceps. Forceps tear through muscle and bleeding again ensues. Wound again clamped. After 3 minutes clamp is loosened and removed and bleeding has ceased. Wound is seen to be lacerated one, measuring about 6 mm. in its longest diameter. Animal seems very weak. In this experiment, while the chloroform was given, the animal seemed to have dyspnœa and during the deep respiratory movements, heart was partly forced out of wound of thorax and hæmorrhage was greater than at any other time.

19-vi-'98. Animal jumps around and eats grass.

24-vi-'98. Animal has just given birth to five fœtuses of about 3 weeks of age.

26-vi-'98. R. 80. External wound healed.

10-vii-'98. Well in every respect.

30-vii-'98. Is gaining weight rapidly.

15-viii-'98. Well.

8-ix-'98. Animal perfectly well.

VI, VII, VIII. Three rabbits weighing 1480, 1720, 1900 gm. respectively. Ether anæsthesia.

In these three animals, the pericardium was exposed but not opened. By means of a needle, the heart muscle was injured by punctures through the pericardial sac and the heart observed. In all three animals, it was noted that—no matter where the wound was made—as the pericardial sac became more and more filled by blood, the heart was pressed more and more firmly against the anterior wall of the pericardial sac and could be plainly seen through it. Heart's action gradually became irregular and slower in each experiment. In VI, heart's action ceased in six minutes; in VII in fourteen minutes; in VIII, in sixteen minutes. The post-mortem conditions in the three animals were almost identical. In each the apex of the heart was found firmly pressed against the anterior wall of the pericardial sac; the heart cavities of each animal were almost empty.

IX. 17-vi-'98. Rabbit. 1590 gm.; P. 148; R. 48. Ether. Internal mammary artery injured and secured only after considerable hæmorrhage. During this procedure, a large rent was made in the left pleura. Thoracic wound at once closed. Animal remained very weak for several days after the experiment.

28-vi.-'98. Killed by blow on neck. Large abscess in left pleural cavity.

X. 17-vi-'98. Rabbit, medium size. R. 48; P. 208. Ether; incision and resection of 4th and 5th ribs in usual manner. Heart is drawn upwards by means of a forceps and during diastole, a wound 2 mm. long is made obliquely in middle of right ventricle. Characteristic arhythmia for three minutes. Hæmorrhage systolic. Heart is allowed to drop back into pericardial sac and wound is compressed with gauze. After several minutes bleeding stops. External wound closed in usual manner after toilette of pericardial sac. Respiration 60; pulse 180. After one hour animal jumps around in its cage as if nothing had been done to it.

23-vi-'98. Skin wound healed.

26-vi-'98. Well in every respect.

28-vi-'98. Killed. Skin wound clean and firmly healed. Pericardium adherent to heart only over the spot where the right ventricle had been wounded. Other organs normal.

XI. 19-vi-'98. Rabbit. 1870 gm., R. 88; P. 224. Ether. Heart, exposed in usual manner, drawn into wound and firmly compressed with anatomical forceps; heart now beats very irregularly. It is then punctured with a scalpel, the blade passing through the wall of the left ventricle, septum and wall of right ventricle.

Enormous systolic hæmorrhage, and some oozing during each diastole. Pericardial sac packed with gauze. After three minutes gauze is removed; hæmorrhage has ceased. External wound closed in the usual manner. Animal is very weak. R. 92; P. 240. Died three hours later. Through an error, the body of the animal was removed before a post-mortem examination had been made.

XII. 19-vi-'98. Rabbit. 1200 gm.; R. 68; P. 240. Under ether, heart is exposed in usual manner. Right auricle punctured at its middle with a "teasing-needle." As the needle is withdrawn, a rent is made in the auricular wall, and a large hæmorrhage ensues. Wound is at once tamponed with gauze. After 4 to 5 minutes tampon is removed; bleeding has stopped. External wound quickly closed. Animal is found dead in its cage the next morning. Post-mortem examination: Pericardial sac filled with clotted blood.

XIII. 20-vi-'98. Rabbit. 1480 gm.; R. 52; P. 192. Ether. Heart, exposed in usual manner, is drawn forwards with forceps, and seven interrupted sutures of fine silk passed through wall of left ventricle. The sutures are 2 mm. apart and do not penetrate into the heart cavity. During the passage of the needle, characteristic arhythmia. Thorax wound closed.

26-vi-'98. Skin wound healed.

10-vii.-'98. Small abscess in line of wound opened. It does not communicate with thoracic cavity.

20-vii-'98. Abscess cavity clean and healed.

22-vii-'98. Killed. There is a small abscess of deeper layers of wall of thorax, extending into the mediastinum but not into the pericardial sac. Parietal pericardium adherent to the visceral layer of greater part of anterior surface of heart. On account of thickened pericardium, it is almost impossible to see the exact line where the sutures had been applied.

XIV. 20-vi-'98. Rabbit. Weight 1400 gm.; R. 52; P. 248. Continuous suture of seven stitches in upper part of right ventricle. Characteristic arhythmia. During the toilette of the pericardial sac, animal awakes from the anæsthesia, struggles violently, and both pleuræ tear. Heart's action at once becomes very slow, and stops after four beats.

XV. 22-vi-'98. Rabbit. 1840 gm.; R. 56; P. 124. Ether. Heart exposed in usual manner. Heart is drawn forwards with mouse-tooth forceps and one fine silk suture is inserted in the lower part of the wall of the left ventricle. By means of this thread, heart is now drawn well forward. When lower part of ventricles is compressed with forceps, characteristic arhythmia is observed. Through the pressure of the mouse-tooth forceps, the cardiac muscle is injured over the lower part of the septum ventriculorum, and the left (?) ventricular cavity is opened. A tremendous systolic hæmorrhage follows. Wound is at once closed by three interrupted sutures. Toilette of pericardial sac. External wound closed.

23-vi-'98. Animal jumps around and eats grass.

10-vii-'98. Well in every respect.

30-vii-'98. Is gaining in weight, now weighs 1920 gm.

10-ix-'98. Is perfectly well.

XVI. 22-vi-'98. Rabbit. 1715 gm.; R. 48; P. 140. Ether. Four interrupted sutures through upper part of wall of right ventricle, 2 mm. apart, and all penetrating into ventricular cavity. Bleeding from needle-punctures is small. Toilette of pericardial sac. Thoracic wound closed in usual manner.

10-vii-'98. External wound healed and animal gaining in weight.

2-viii-'98. Killed. Post-mortem examination: External wound healed. Small abscess under skin containing about one drop of pus. Parietal pericardium adherent to the visceral layer only over the situation of the sutures. Surface of heart has smooth normal appearance.

XVII. 23-vi-'98. Rabbit. 1000 gm.; R. 68; P. 184. Ether. Six interrupted silk sutures in upper part of wall of left ventricle. Suture includes only pericardium and superficial layers of myocardium. There is almost no bleeding. Characteristic arhythmia absent.

10-vii-'98. Wound healed.

2-viii-'98. Animal has increased in weight, now weighs 1400 gm.

3-viii-'98. Killed. Post-mortem: External wound clean and healed. Pericardial sac closed and adherent to visceral pericardium of greater part of anterior surface of heart. Heart muscle, on section, looks normal.

XVIII. 23-vi-'98. Rabbit. 1920 gm.; R. 100; P. 220. Ether. Wound 3 mm. long and 3 mm. deep made over the middle of the septum of the ventricles on the anterior surface of the heart. Hæmorrhage systolic. Wound closed by five fine silk sutures. External wound closed in usual manner.

26-vi-'98. Animal seems well.

1-vii-'98. Is found dead in its cage. Post-mortem examination shows large abscess in thoracic wall. Wound in heart-wall firmly closed.

XIX. 24-vi-'98. Rabbit. 1580 gm.; R. 76; P. 200. Ether. Usual operation for exposure of heart. During this procedure, the internal mammary artery was cut; it was however ligated before much bleeding had occurred. One suture in the middle of the wall of the left and another in the middle of the wall of the right ventricle. Continuous suture of six stitches in the lower part of the wall of the right auricle. All the sutures penetrate into the heart cavities. The bleeding from the needle-punctures is small and is immediately arrested when the suture is drawn tight and tied. Heart's action rapid and irregular.

26-vi-'98. R. 92. Animal appears to be well.

10-vii-'98. R. 92. External wound healed.

22-vii-'98. Killed. Wound in auricle firmly healed and parietal pericardium adherent to it. All other organs normal.

XX. 24-vi-'98. Rabbit. 1380 gm.; R. 88; P. 200. Ether. Penetrating wound, 3 mm. long over septum of the ventricles. Hæmorrhage systolic. Four interrupted sutures. A second wound, 4 mm. long, is made in apex of the heart, penetrating into the left ventricle. It is closed by four interrupted silk sutures. Heart's action rapid but strong.

26-vi-'98. Is well. R. 92.

10-vii-'98. Skin wound firmly healed. R. 80.

20-viii-'98. Animal is gaining in weight. Now weighs 1500 gm. Killed. Wounds in heart-muscle are firmly cicatrized and the parietal pericardium firmly adherent to visceral layer over them.

XXI. 24-vi-'98. Rabbit. 1600 gm.; R. 68; P. 200. Ether. Heart exposed in usual manner. A tobacco-pouch suture is applied transversely around the ventricles, so that the lower third of the ventricles is below the suture. This is then drawn tight and tied. The part of the ventricles that is thus cut off from the rest of the heart continues to contract, but one has the impression that each contraction lags behind a moment. The action of the whole heart remains good. The portion of the ventricles below the suture is then excised and the pericardium united over the cut surface. Heart's action remains good and there is no bleeding. Toilette of the pericardial sac. External wound closed in usual manner.

25-vi-'98. Animal seems to be well.

30-vi-'98. External wound healed.

10-viii-'98. Now weighs 1800 gm.

10-ix-'98. Is perfectly well.

20-ix-'98. Killed.

XXII. 25-vi-'98. Rabbit. 1410 gm.; R. 100; P. 280. Ether. A penetrating wound, one centimetre long, is made in the wall of the right ventricle during a diastole. A tremendous hæmorrhage follows which cannot be totally controlled by compression of lips of wound with artery forceps. Five interrupted sutures of silk close the wound and control the bleeding. Several of these sutures, having been tied during systole, tear out through the muscle. Fresh sutures are passed and these are tied during diastole. Every one of these holds.

26-vi-'98. Very weak and will not eat.

27-vi-'98. Found dead in its cage. Post-mortem examination: Pericardial sac filled with clotted blood. The lower two sutures in heart-muscle, having been applied and tied during a systole, have torn through the muscle and the animal has gradually bled to death.

XXIII. 25-vi-'98. Rabbit. 1510 gm. Ether. Incision one-half centimetre long in wall of right ventricle near septum. Closed by continuous suture, each stitch of which is drawn tight during a systolic contraction of the ventricles. Several of the stitches tear through the heart-muscle and enormous hæmorrhage ensues. Heart's action becomes very slow and irregular and soon stops.

XXIV. 27-vi-'98. Rabbit. 1750 gm.; R. 48; P. 220. Ether anæsthesia. A continuous suture was applied to the wall of the left ventricle, beginning at the base and extending to the apex of the ventricle, and the suture is there continued along the wall of the right ventricle from apex to base. Sixteen stitches passed, some of which penetrate into heart cavity, others pass through the pericardium and superficial layers of myocardium only. The bleeding from the superficial needle punctures is much smaller than that from the penetrating punctures. Characteristic arhythmia each time the needle is passed. Several of the stitches tear out of the muscle and have to be applied a second time.

28-vi-'98. Animal appears to be well.

10-vii-'98. Animal has been perfectly well. Skin wound firmly healed.

10-ix-'98. Animal has been steadily gaining in weight. Killed by blow on neck. Post-mortem examination shows that heart's surface is smooth and free, except along line of suture where visceral pericardium is considerably thickened.

XXV. 27-vi-'98. Rabbit. 1570 gm.; R. 64; P. 204. Ether anæsthesia. Right and left appendix auriculæ tied off and cut off. Toilette of pericardial sac.

28-vi-'98. R. 64; P. 220.

10-vii-'98. Seems perfectly well.

29-vii-'98. Animal looks sick.

30-vii-'98. Died. At the post-mortem examination the heart wounds cannot be found, the pericardium is very much thickened over the whole heart. Macroscopically and microscopically heart-muscle is normal. Marked hepatic coccidiosis.

XXVI. 27-vi-'98. Rabbit. 1840 gm.; R. 64; P. 200. Ether. A temporary ligature tied transversely around ventricles so as to include about one-third of the ventricles below the ligature. A small scalpel is then made to pass through the whole thickness of the heart below the ligatures, thus penetrating the wall of left ventricle, the septum between the ventricles and the wall of the right ventricle. The scalpel is then made to cut downwards toward the apex and through it, so that this lower part of the heart is divided into two and both ventricular cavities opened. On account of the provisional ligature, there is no bleeding. The wound, about one centimetre in length is closed by eight interrupted sutures. Provisional ligature then removed. There is no bleeding. Toilette of pericardial sac. Suture of external wound.

10-vii-'98. External wound healed. Animal is well in all respects.

20-ix-'98. Now weighs 2020 gm. Killed. Post-mortem examination shows that heart wound is firmly healed and parietal pericardium is adherent over it.

XXVII. 27-vi-'98. Rabbit. 2000 gm. Same operation as in Experiment XXVI.

10-vii-'98. External wound healed. Animal well.

20-vii-'98. Is losing in weight and looks badly.

29-vii-'98. Found dead in its cage. Large abscess in mediastinum. It does not communicate with heart wound, which latter is firmly cicatrized.

XXVIII. 30-vi-'98. Rabbit. 2160 gm. Same operation as Experiment XXI. After operation animal was very weak and died forty hours later.

XXIX. 30-vi-'98. Rabbit. 2440 gm.; R. 64; P. 220. Ether. Wound 6 mm. long, penetrating left ventricle. Systolic hæmorrhage. Wound closed rapidly by four interrupted sutures. Another wound 3 mm. long and penetrating into cavity of right ventricle made and closed with two sutures. Right appendix auriculæ tied off and cut off below ligature.

8-vii-'98. External wound healed. Animal well.

5-viii-'98. Is gaining in weight.

6-ix-'98. Is very well. To-day gave birth to four young ones.

XXX. Rabbit. 1450 gm. Same operation as in Experiment XXI except that somewhat more than one-third of ventricles was excised. Animal very weak and died eighteen hours after the experiment.

XXXI. 7-vii-'98. Rabbit. 2050 gm. Same operation as in Experiment XXVI. Eight sutures applied superficially in the usual manner. There is no bleeding, and heart's action remains strong and good.

21-vii-'98. Animal has remained well. Killed. Heart wound healed. Scar is broad and thin and bulges a little.

XXXII. 8-vii-'98. Rabbit. 2230 gm. Operation in all respects similar to Experiment XXIV. During the manipulations several of the stitches tore out and animal lost considerable blood. It remained very weak and died ten hours later.

XXXIII. 8-vii-'98. Rabbit. 2450 gm.; R. 64; P. 220. Operation as in Experiment XXIV, except that the continuous suture consisted of 24 stitches.

10-viii-'98. Is well; external wound healed.

10-ix-'98. Well and gaining in weight.

XXXIV. 11-vii-'98. Rabbit. 1850 gm. The same method of operation as in Experiment XXI except that only one-fourth of the ventricles is excised. This animal remained well and at the expiration of seven days, when the skin wound was healed, it was killed and the heart removed for microscopical examination.

XXXV. 12-vii-'98. Rabbit. 1300 gm. Operation similar to Experiment XXI.

10-ix-'98. Animal has remained well and is gaining in weight.

XXXVI. 13-vii-'98. Rabbit. 1200 gm. Ether. Appendix auriculæ and about one-fourth of the left auricle tied off by a ligature thrown around it and cut off.

10-ix-'98. Animal has remained well.

XXXVII. 13-vii-'98. Rabbit. 1200 gm. Operation as in Experiment XXI. Killed after four days for microscopical study of heart.

XXXVIII and XXXIX. 18-vii-'98. Weight of each rabbit 1100 grammes. Each operation as in Experiment XXVI with similar observations made as in that experiment.

10-ix-'98. Both animals have remained well and have gained in weight.

XL. 20-vii-'98. Rabbit. 2000 gm. Operation as in Experiment XXI.

20-viii-'98. Is well and gaining in weight.

XLI. 20-vii-'98. Rabbit. 1500 gm. Same operation as in XXIV except that a part of both auricles was included in the continuous suture.

15-viii-'98. Skin wound healed and animal seems to be well.

20-ix-'98. Killed. Heart surface smooth. No adhesions between parietal and visceral layers of pericardium.

XLII. 20-vii-'98. Rabbit. 1850 gm. Operation as in Experiment XXVI. The provisional ligature slipped, an enormous hæmorrhage ensued, which caused the death of the animal in a few moments.

XLIII to XLIX. In these experiments, wounds as in XXI, XXIV and XXVI were made and the animals killed in one to seven days for histological purposes.

L. Medium-sized dog. Morphine and ether. During the manipulations incident on exposure of the pericardial sac, but pleuræ were opened and before artificial respiration could be begun, the heart stopped beating.

LI. Medium-sized dog. Morphine, gr. ¼. Ether anæsthesia. Tracheotomy performed and cannula inserted into trachea and made ready for connection with apparatus for artificial respiration. Fourth, fifth and sixth ribs exposed by longitudinal incision along left border of sternum. About 1½ inches of each of these ribs excised. Left pleura incised and pericardial sac quickly grasped and drawn up into wound. It was then sutured to the muscles around the edges of the thoracic wound and opened by a longitudinal incision. The heart was then manipulated in a manner similar to that described for the rabbit's heart. It was grasped with a forceps, punctured with needles, a ligature thrown around the lower portion of the ventricles and tied tightly, etc. The results obtained were analogous to those in rabbits. The same arhythmia of the heart, the same irritability of the pericardium and endocardium was present. Hæmorrhage from the needle punctures was *less* than in the rabbits. When the pericardium alone was penetrated, there was no bleeding at all. When the needle was made to penetrate into the ventricular cavity, the hæmorrhage was somewhat larger but ceased quickly. The right ventricular wall was punctured with the needle; the bleeding was larger than on the left side but also soon ceased. A continuous suture from base to apex of right ventricle was made. The application of the suture was easier than in the smaller animals. Toilette of the pericardial sac, after which the latter was closed by continuous silk suture, and the muscles and skin sutured. Tracheotomy wound closed and iodoform collodion dressing applied to both wounds.

Two hours later the dog, though still under the influence of the morphine, was attempting to run around. The animal was found dead in his cage next morning, ten hours after operation. Post-mortem examination showed that from some cause, perhaps the restlessness of the animal, both pleuræ had been torn and both pleural cavities contained a small quantity of bloody serum.

LII. Large Dog. Heart exposed in the same manner as in Experiment LI. A wound 1.5 centimeters long, penetrating into the left ventricular cavity was made. An enormous hæmorrhage ensued. The wound was closed by pressure with artery forceps. The edges of the wound were then united by means of two deep, buried, and two superficially placed sutures. All the sutures were tied during systole. Pericardial sac and external wound closed as in LI. Death eight hours later. Post-mortem examination shows wound 1.5 ctm. in left ventricular wall to be firmly closed. Left

pleural cavity half-filled with fluid blood. When the superficial sutures were cut and the deeper parts of the heart-wound were examined, the buried sutures were found to have partially torn out through the heart-muscle.

LIII. Medium-sized Dog. Heart exposed by a procedure similar to that of Experiments LI and LII. Interrupted sutures were applied to numerous points on both ventricular walls in order to investigate the best method of applying and tying the sutures. Suture through whole thickness of left wall near base. This suture tears out at once, and necessitates the applications of two superficial sutures penetrating only the pericardium and superficial layers of myocardium. These hold. Three sutures passed through left ventricular wall and three through right ventricular wall. Two of the sutures on each side (a, b) tied during systole, the remaining one (c) during diastolic relaxation of the ventricles. Of these, two of the former (a, b) tear through the heart-muscle. Two sutures applied to replace these and tied in diastole; they hold. Pericardial layer incised for about two centimetres. The bleeding is considerable at first but ceases after compression with gauze. An attempt was made to observe the effects of heart tamponade. An incision 1 cm. long was made in the wall of the right ventricle and the opening in the pericardial sac rapidly closed by means of artery forceps. The bleeding was so great, however, that the sac burst in its lower and posterior part and the animal died in a few moments from the blood that escaped into the left pleural cavity.

Six experiments on rabbits, in which observations on the physiological irritability of the heart-muscle were made are not described in order to save repetition of what has been detailed in Part II, Series I, page 491.

A CASE OF ACUTE ENDOCARDITIS CAUSED BY MICROCOCCUS ZYMOGENES (NOV. SPEC.), WITH A DESCRIPTION OF THE MICROORGANISM.*

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Clinical History. September 14, 1898, a German, aged 37 years, was admitted to the service of Dr. Osler, complaining of fever which had persisted since early in July. The history of the patient's family and his occupation were of no importance. In 1889 the patient was confined to bed three weeks with "rheumatic fever," though no joint symptoms were recalled. Otherwise the history was that of a healthy man of good habits.

July 4, a severe cold was contracted, fever appearing on the 6th and persisting until early in August when it subsided but reappeared about the middle of the same month, and for this relapse in supposed typhoid fever the patient was sent to the hospital. Severe frontal headache, stiffness and pain in the right shoulder joint, epistaxis, loss of strength, anæmia, and loss of 26 pounds in weight were noted during July and August.

Examination on admission showed a well-built, emaciated man, with a moderate grade of anæmia, and a temperature of 103° F. There was decrease in the area of cardiac dulness, and a pure diastolic murmur was detected over the body and base of the heart, suggesting disease of the aortic valves. The pulse also indicated incompetency of these valves. Over the abdomen were scattered a few erythematous spots thought at the time to be the rose spots of typhoid fever. The spleen was large, readily palpable and rather firm. The right shoulder joint which in July had been painful and stiff was now apparently normal. The urine showed a well-marked Ehrlich's diazo-reaction and a faint trace of albumin. During the first ten days in the hospital, anæmia, emaciation, fever, enlarged spleen and the cardiac condition of aortic incompetency

* A preliminary communication appeared in the Bulletin of the Johns Hopkins Hospital, 1899, x, p. 46.

were the most striking symptoms. At times stiffness and pain about the right shoulder joint were complained of, but there were at no time signs of inflammation about the joint.

The high temperature of 103-104° F. and a leucocytosis of 18,000 persisted. A suggestive Widal reaction also was present.

Though in a satisfactory condition on the evening of September 23, the next morning the patient appeared much more seriously ill. Delirium of the night previous had given way to stupor; anæmia was of extreme grade; the extremities were cold; and the appearance one of collapse. The lungs were quite clear but the heart, dilated to twice its previous size, had become rapid and irregular in action, and the physical signs of aortic disease were no longer recognizable. On the afternoon of September 23, the maximum cardiac impulse was readily located in the 5th left interspace, 9 cm. from the midsternal line, and the area of relative and absolute cardiac dulness was less than normal; on the morning of the 24th the maximum cardiac impulse was in the 6th left interspace 12.5 cm. from the midsternal line and the area of relative and absolute cardiac dulness had increased to twice that of the normal. During the night of September 24, the cardiac condition had somewhat improved and endocardial murmurs and a palpable thrill appeared at the apex suggesting both mitral and aortic disease.

At this time agar plate cultures from the blood were prepared on which within 48 hours grew colonies of what appeared to be a short-chained streptococcus, which subsequently on various media proved to be a micrococcus occurring mostly in pairs.

There subsequently occurred repeated attacks of collapse from cardiac failure up to the time of the patient's death on October 3, 1898.

October 1, three days before death, blood cultures were again prepared. On these cultures there grew the same micrococcus occurring mostly in pairs, the growths on various media corresponding to those obtained from the cultures prepared September 24.

Autopsy. Performed on the day of death, the body having been kept on ice and showing no evidences of post-mortem change. Description will here be given of only those organs which were the seat of pathological change.

Anatomical diagnosis. Subacute and acute ulcerative endocarditis of aortic and mitral valves; acute splenic tumor; septic infarctions in spleen and kidneys; embolic abscess in intestinal wall; bronchopneumonia; chronic diffuse nephritis of moderate degree.

Body emaciated, 184 cm. long; rigor mortis slight. Abdominal muscles of a deep red color; no excess of peritoneal fluid; peritoneal surfaces

smooth and glistening; a few old pleuritic adhesions; pericardial layers smooth.

Heart distended with liquid blood; weight 420 grammes. Nothing abnormal in the right heart. On the left side the mitral valve showed slight old thickening and in the middle of the edge of the aortic segment was a small, dark-red, fresh vegetation; many of the chordæ tendineæ had been ruptured, the ends of the ruptured cords being coated with fibrinous deposits; others were markedly thinned in the median portion, the adjoining parts being much thickened by fresh vegetations. The aortic valves were matted together by the exuberant vegetations which had formed upon them; a prolongation on the anterior coronary segment formed a flattened plate, which was apparently in part at least a mass of organized tissue, perforated by a hole about 2 mm. in diameter; the posterior coronary segment was surmounted by a rough mass of vegetations; the mitral segment was similarly covered with vegetations through which a ragged perforation was evident. From a point on the ventricular wall at the base of the posterior coronary segment to the base of the aortic segment of the mitral valve there ran a cord, like a moderator band, thickened in its median portion by beaded vegetations and entangled in the clots formed about the aortic vegetations. There were fresh vegetations also on the ventricular wall and on the wall of the aorta just above the valves.

Spleen, bound up in adhesions with the diaphragm and parietal abdominal wall, was much enlarged and very soft so that on attempting to remove it the capsule was ruptured in several places. Weight 470 grammes. The surface was peculiarly discolored over irregular areas which occupied a large portion of the capsule. Beneath some of these the substance was firmer than normal and on section elevated above the surrounding tissue, such areas corresponding to firm yellowish-white masses extending into the splenic pulp and sharply marked off by hæmorrhagic zones. All degrees of softening of such masses were present and the larger discolored portions of the capsule proved to form merely thin walls covering irregular cavities in the spleen filled with purplish-brown grumous fluid. These cavities also were delimited from the surrounding tissue by mottled zones of red and white. The relatively normal splenic pulp between the areas of infarction was much swollen and the Malpighian bodies and trabeculæ could be made out only with great difficulty.

Kidneys also presented superficially areas of purplish and grayish discoloration, of varying form and size, sharply demarcated from the surrounding cortex by lines of congestion and hæmorrhage. On section

these areas were seen to correspond with somewhat wedge-shaped masses varying in consistence and color. The smallest and evidently the freshest of these were firmer than normal, yellowish-white and elevated above the surface of the adjacent tissue; the larger and older masses were much softened and of a grayish-purple color throughout. In addition, the kidneys presented evidences of moderate chronic diffuse nephritis.

In the lower third of the *ileum* one of the lymphatic nodules in the mucosa appeared much swollen and reddened with a zone of hæmorrhage round about it. This nodule was quite firm and elevated several millimetres above the surface of the mucosa.

The left *lung* showed small bronchopneumonic patches throughout the lower lobe.

Microscopic examination. Sections of the diseased organs showed both acute and subacute morbid processes. Sections of the cardiac valves and vegetations showed a considerable curling and twisting of the valve proper. Thrombus masses surmounted the valves and formed the support for a fairly advanced growth of granulation tissue. The more superficial portions of the vegetations, however, consisted of platelets, fibrin and leucocytes, together with myriads of micrococci which, stained by Weigert's or Gram's method, formed dense bluish-black masses throughout the thrombus. Smear preparations from the vegetations showed the micrococci apparently unmixed with any other organisms and occurring often within leucocytes.

Sections through the discolored and softened areas in the kidney showed the typical picture of septic infarction with complete necrosis of the epithelium and the invasion of many polymorphonuclear leucocytes. The blood-vessels near the apex of the wedge were in many instances plugged with masses of micrococci. The firmer whitish masses both in the kidney and spleen were simple anæmic infarctions.

The small nodule in the ileum showed on section a mass of lymphatic tissue in the centre of which a small artery was completely occluded by a dense hyaline thrombus; about the vessel was an accumulation of polymorphonuclear leucocytes and nuclear fragments, together with a considerable exudation of fibrin; the neighboring vessels were also congested.

Cultures. Agar cultures in Petri dishes were made from the heart's blood, valvular vegetations, gall-bladder, splenic and renal infarctions, and other parts, and in all of these there appeared numerous, minute, pin-point, somewhat opaque, white colonies in the depths of the medium, and corresponding superficial colonies. The latter were also small, rarely exceeding the size of a pin's head. They were round in outline,

smooth, glistening, transparent, almost colorless or, on microscopical examination, perhaps slightly brownish, and slightly elevated above the surface of the medium. No other kind of colonies appeared on the Petri plates.

From the deep and superficial colonies obtained from the different organs a single species of microorganism was isolated and studied in all of its essential characters.

DESCRIPTION OF THE MICROORGANISM.

Morphology. Coverslips prepared from the original colonies and from the secondary tube-cultures showed a micrococcus in every way identical morphologically with that found in smears from the cardiac valves and with that isolated from the blood during life.

The organism is an extremely minute micrococcus, often somewhat elongated or elliptical in outline. It is much smaller than the Micrococcus lanceolatus. It occurs often singly, often in masses, but by far most frequently in pairs, two or more pairs being sometimes united into short chains, and indeed in cultures made in a hanging drop of bouillon chains of twenty or more members may occasionally occur, although in such a drop the usual grouping is in pairs. In the pairs or short chains the longer axis of the coccus is often transverse to that of the chain.

No motility is to be observed in the hanging drop other than the oscillatory motion common to all fine particles in suspension.

The organism stains well with the ordinary aniline dyes and by Gram's method remains deeply stained. No evidence of the presence of a capsule has been obtained either in cultures or in the tissues.

Cultural characteristics: Agar. Smears made in slanted agar tubes give a rather profuse, thin, slightly elevated growth along the line of the smear. The growth is somewhat moist and glistening, almost colorless; by reflected light, pale grayish-white; by transmitted light, transparent and of a somewhat smoky or brownish tint, old cultures showing this smoky discoloration more distinctly. The growth is not always, however, a diffuse flat expanse, but is often made up of a conglomeration of minute dew-drop-like colonies. The margins are somewhat crenated by the presence of larger, and sometimes

discrete, glistening colonies, slightly more elevated than those nearer the centre. Superficial, discrete colonies on agar may somewhat exceed one millimetre in diameter.

Glycerine agar. Cultures on glycerine agar grow more profusely but in other respects are identical with those on plain agar.

Ascitic-fluid agar. On ascitic-fluid agar the growth is distinctly more profuse and opaque than upon plain nutrient agar.

Glucose agar. The growth along the stab in glucose agar is moderately profuse but neither in stab cultures nor in cultures made in liquefied glucose agar, which is then allowed to solidify, is there any production of gas.

Bouillon. Broth becomes slightly clouded after twenty-four hours' growth, but in the course of three or four days the organisms settle to the bottom and form a whitish sediment leaving the overlying fluid clear. No indol is produced.

Sugar bouillon. In glucose and in lactose litmus bouillon (Smith's) in Smith's fermentation tubes acid is produced without gas, the medium becoming turbid in both arms and decolorized in the closed arm.

Potato. On potato, minute colonies appear and in about 36 hours become elevated, moist, of a somewhat dirty white color and pasty consistence, and after 72 hours confluent. Later the growth becomes rather dry and brownish. Occasionally there is no visible growth on potato.

Gelatine. In stab cultures in gelatine a somewhat opaque, granular growth extends along the line of stab and after about 36 hours a slight cupping appears at the surface and extends slowly downward, producing a tubular area of liquefaction which gradually involves the whole medium. Liquefaction is slower than in the case of Staphylococcus pyogenes aureus.

In gelatine plates the organism forms small pale granular colonies, yellowish by transmitted light, which float in small areas of liquefied gelatine.

Blood serum. On coagulated blood serum the growth is profuse and similar to that on agar, although perhaps somewhat less trans-

parent; along the line of smear the medium becomes somewhat translucent and is depressed, forming a definite groove below the surrounding surface. This change is similar to that observed in cultivations of Bacillus subtilis on blood serum. This partial liquefaction is accompanied, as a rule, with the production of a clear fluid and the breaking down of the solid medium.

Milk. The most characteristic cultural features of this organism appear in milk. In litmus milk of neutral reaction the immediate result of the growth is decolorization of the litmus within a period of four hours, a thin blue layer remaining at the surface. This decolorization is evidently due to deoxidation as the color returns to a certain extent on shaking the fluid with air, the returning color being, however, distinctly nearer to red than the original. Within twenty-four hours the milk becomes quite firmly coagulated, with the bluish or reddish layer still at the surface and the absence of color below. Next in time the upper layers of the coagulum become translucent and are gradually changed into a somewhat turbid fluid which is definitely red in the more superficial part and yellowish below. This softening and liquefaction of the coagulum progresses day by day, the layer of red fluid increasing in depth and the coagulum being transformed into a flocculent granular material floating in a yellowish liquid; finally, after several days, the whole coagulum is transformed and the red color extending into the depths stains the precipitate throughout; settling of this precipitate to the bottom of the tube at last leaves a clear straw-colored supernatant fluid overlying a dark red sediment.

These changes produced in litmus milk are absolutely constant and serve to distinguish this micrococcus sharply from related members of the pyogenic group of cocci.

Production of enzymes. Attempts were made to determine the nature of some of the metabolic products of the micrococcus.

Grown in sugar-free bouillon the reaction of the culture is alkaline, whereas in ordinary sugar-containing and lactose-containing bouillon it is acid. The acid reaction of milk cultures is, therefore, doubtless due to the production of acid by the decomposition of the milk sugar.

Experiments were made to determine whether in addition to the

formation of acid a milk-curdling ferment (Labferment, rennin, pexin) was produced in sufficient quantity to coagulate milk. Milk cultures, which had reached the stage of coagulation, were passed through a Pasteur filter to remove the bacteria and the resulting clear filtrate was added in small quantity to sterilized litmus milk. No appreciable change in the reaction of the milk followed this addition, but within 36 hours there was produced a coagulum quite as firm as that caused by the action of the living micrococci themselves. No decolorization nor acidification of the medium occurred. In the course of time the coagulum retracted somewhat, leaving a clear fluid overlying it. This experiment establishes the existence of a rennin-like ferment among the products of the micrococcus. The solution of the coagulated casein, as well as the liquefaction of gelatin and blood serum, indicate the formation of a proteolytic ferment related more or less closely to enzymes concerned in the digestion of proteids. The sterile filtrate, obtained by passing through a Pasteur filter milk cultures in which the liquefaction of the coagulum is in progress, was added to sterile milk and to nutrient gelatine for the purpose of observing the production of these changes without the presence of the organisms themselves. In these experiments the curdling of the milk proceeded as described above without much change in the reaction. On standing in the thermostat, this coagulum has of course a tendency to contract, but a further diminution in its size by the softening and liquefaction of its superficial layers is quite evident. The liquefaction is, however, not so complete as when the living organisms are present.

When a few drops of the filtrate are dropped upon the surface of sterile nutrient gelatine, this medium becomes slowly liquefied with the production of a clear fluid. Control inoculations in bouillon, milk and agar from these tubes of coagulated and peptonized milk and of liquefied gelatine, gave uniformly negative results, and coverslips from them showed no organisms.

The conclusion, therefore, is justified that this micrococcus produces both milk-curdling and proteolytic enzymes, separable from the bacterial cells and capable, when thus isolated, of producing their characteristic effects in milk and in gelatine.

Relation to oxygen. The micrococcus is a facultative anaërobe, the cultures grown in Buchner jars presenting quite as luxuriant a growth as those kept in the open air. Grown in hydrogen in a Novy jar, however, the colonies are rather more scanty and thinner.

Vitality. The organism is very hardy and tenacious of life. Agar cultures which stood in the laboratory from October until February and which were very much dried out furnished a profuse growth on a fresh agar tube. In this respect it obviously stands in marked contrast to Micrococcus lanceolatus which can be kept alive only with much difficulty.

Moderate variations in temperature seem to have no deleterious influence on the micrococcus. Indeed, it can withstand a temperature higher than that which is fatal to many micrococci. In order to fix the thermal death-point, portions of a bouillon suspension of the organism were sealed in Sternberg bulbs and heated for a period of five minutes each, to different temperatures. After exposure for five minutes to a temperature of 65° C., no organisms grew when the bouillon was transferred to the surface of an agar slant. After heating to 62°, only one or two colonies developed, but exposure to a temperature of 60° for five minutes produced hardly any retarding effect upon the growth of the organisms.

To antiseptics, such as carbolic acid and chloroform water, the micrococcus is considerably resistant, a relatively large precentage of antiseptic being required to kill all of the organisms in bouillon cultures.

Pathogenesis. The ordinary laboratory animals were inoculated in various ways with cultures and suspensions of the organism.

White mice. These animals, inoculated intraperitoneally with various quantities of thin suspensions of the organisms in bouillon, died in the majority of the experiments after periods varying from seven hours to three or four days, with evidences of general infection. Autopsy showed always a much swollen spleen and sometimes reddened lymph glands. The micrococci were recovered in pure culture from the heart, spleen, liver, and kidney. The dose given varied from 0.3 to 0.7 cc. of the suspension.

White mice were inoculated with a loopful of the solid culture also subcutaneously, but although they died after several days, the organism was not recovered from the organs. Inoculated subcutaneously with relatively large quantities of the bouillon suspension (0.8 to 1.8 cc.) they died within four to ten days, the organs showing the same changes as after intraperitoneal inoculation and yielding the cocci again in pure culture. No macroscopic lesion of the muscles near the seat of subcutaneous inoculation was visible.

Wild mice proved to be distinctly less susceptible than the white, although occasionally they succumbed to intraperitoneal inoculation with general septicæmia.

Guinea-pigs, *white rats* and *pigeons* showed very little susceptibility to inoculation.

Rabbits. A number of rabbits were inoculated, for the most part intravenously, but also intrapleurally and intraperitoneally. The latter modes of inoculation were followed by recovery of the animal. Indeed, rabbits appear, in general, rather resistant to this organism, since of eight rabbits inoculated only one developed a general septicæmia. Two of the rabbits showed at the site of inoculation at the time of death, abscesses which contained the micrococcus, and from one of them, as well as from the animal which died with a general infection, the organism was recovered from the contents of the distended urinary bladder.

One of the rabbits received 2.5 cc. of a thick bouillon suspension, containing particles of sterilized potato, into the ear vein. This rabbit became very ill and died after five and a half days, the micrococcus being recovered in pure culture from the blood and all the organs. The condition of the heart was most interesting. The mitral valve showed the presence of two quite firm vegetations, one on each leaflet, that on the aortic leaflet being the larger and measuring about 2 mm. in diameter. The thrombus mass was situated very near the edge of the valve and there was a propagated dark red clot running upward toward the base of the valve on the auricular surface. The smaller vegetation on the opposite leaflet of the mitral valve was situated somewhat nearer the base of the valve, and also on the

auricular surface. The papillary muscles appeared opaque and yellowish. The other valves, as well as the musculature, were apparently normal. Smears from the mitral vegetations showed the presence of the micrococci, unmixed with other organisms, and cultures from these vegetations gave a pure growth of the micrococcus.

Thus of seven rabbits inoculated three showed no ill effects, two died after several days with no apparent lesions at autopsy and no evidence of general infection (from these the organism was not recovered), one died of an intercurrent affection with a small abscess containing the micrococcus at the point of inoculation, one died with a large abscess at site of inoculation, the organism being recovered from the abscess and the distended urinary bladder, and finally one rabbit died with a general septicæmia, together with an *acute vegetative mitral endocarditis*, from which the organism was recovered in pure culture.

Dogs. Two dogs were also inoculated. One of these showed no ill effects, although 10 cc. of a thick suspension, containing particles of potato, were injected into the jugular vein; after sixteen days this animal was killed but at the autopsy the organs appeared quite normal and cultures from all parts were sterile.

In the case of the second dog, Rosenbach's procedure was adopted. The left carotid artery was opened under aseptic precautions, the aortic valves were torn through and lacerated by means of a long probe passed down the carotid, and the artery was then ligated on both sides of the opening. Two cubic centimetres of a thick suspension of the micrococcus in bouillon were then injected into the left jugular vein which was then ligated and the wound of the skin was closed with sutures. The dog appeared rather ill for a few days, but evidently improved slightly after the fourth day. Immediately after the operation there was detected a loud and distinctly diastolic murmur which persisted until the seventh day when the dog was killed with chloroform and an autopsy performed.

The spleen was found enlarged, congested and partly covered with a fibrino-purulent exudate. The other organs were macroscopically apparently normal with the exception of the heart. The right side of

the heart was normal. The aortic valves were the seat of an *acute and subacute endocarditis.* The probe had passed into the sinuses of Valsalva and had penetrated into the muscular tissue of the septum ventriculorum, running down 1.5 cm. just to, but not piercing, the endocardium over the septum. On section the muscle about this area was grayish and translucent in appearance with grayish opaque dots scattered over the translucent area. A second injury had occurred in the sinus behind the mitral segment of the aortic valve, the probe perforating the base of the valvular segment and passing into the ventricular cavity near the base of the mitral valve. About this opening there were on both surfaces of the valve granular translucent vegetations which extended upon the mitral valve and also upon the intima of the aorta just above the aortic valves. Coverslips from the vegetations and necrotic muscle showed numerous micrococci in pairs, many of them within leucocytes. Cultures from the same locations gave a pure growth of the micrococcus, as did cultures from the blood, liver, spleen, and kidney.

Toxine. Attempts were made to obtain a toxine from cultures of this organism, but so far with little success. A priori, it would not be expected that an organism so closely resembling in its general properties the pyogenic group of cocci would produce a toxine of high virulence. The wide dissemination of the cocci observed in the cases of general infection would also point to the same inference, and this suggestion is supported by the few experiments thus far made relating to this point.

A culture, made in a large quantity of sugar-free bouillon and allowed to grow in a Roux flask for three weeks, was killed with chloroform. Ten cubic centimetres of this killed culture, after evaporation of the chloroform, were injected into the ear vein of a rabbit, a control rabbit receiving a similar injection of the same quantity of sterile sugar-free bouillon. Neither rabbit showed elevation of temperature or any other ill effect following the injection.

SUMMARY.

From a case of acute endocarditis of the aortic and mitral valves with infarctions in the spleen and kidneys a micrococcus was twice

isolated in pure culture from the blood during life and was demonstrated after death both microscopically and in pure culture in large numbers in the valvular vegetations, the infarctions and other parts. No other species of microorganism was found.

This micrococcus is very small, occurs mainly in pairs, sometimes in short chains, stains by Gram's method, grows in small, pale, grayish-white colonies on gelatine and agar, at first clouds bouillon, which then becomes clear with a whitish sediment, does not produce gas in glucose media, liquefies gelatine slowly and to some extent also blood serum, and is especially characterized by its behavior in milk, which it acidifies, coagulates and subsequently liquefies. It produces a milk-curdling ferment and also a proteolytic ferment, each of which is separable from the bacterial cells. It remains viable for months in old cultures and is tolerably resistant to the action of heat and antiseptics.

The micrococcus is pathogenic for mice and rabbits, causing either abscesses or general infections. Typical acute vegetative endocarditis was experimentally produced by intravenous inoculation of the organism in a rabbit and a dog, and the cocci were demonstrated in pure culture in the vegetations and other parts of these animals after death.

Although the micrococcus here described has some points of resemblance to the pneumococcus and Streptococcus pyogenes on the one hand and to the pyogenic staphylococci on the other, it is readily distinguished from each of these species by cultural features which have been described and which are so obvious that the differentiation of these species from our micrococcus need not be discussed in detail. We have searched through the records concerning microorganisms described in association with endocarditis and other diseases, as well as those isolated from water, soil and other sources, and have been unable to find a description of a micrococcus identical in all particulars with that here described. Such points as staining by Gram, liquefaction of gelatine, coagulation and peptonization of milk, served singly or in combination to distinguish our micrococcus from other forms which

in some respects might resemble it.* We feel justified, therefore, in recognizing this organism as a new species and from its fermentative properties propose for it the name "Micrococcus zymogenes."

Micrococcus zymogenes must be added to the already considerable list of bacteria which have been found as the specific infective agents in endocarditis. That it was the cause of this affection in our case was conclusively demonstrated by its repeated isolation in pure culture from the blood during life, by its presence in pure culture and large numbers after death in the cardiac vegetations, the infarctions, and other parts of the body, and by the experimental proof of its pathogenic properties, and notably its capacity to produce vegetative endocarditis by intravenous inoculation in animals.

* Dr. Norman MacL. Harris in this laboratory isolated in May, 1898, from material removed from an old cesspool in Baltimore, which had not been evacuated for twenty-two years, a micrococcus which in morphological and cultural characters is so similar to Micrococcus zymogenes that we at present believe the two organisms to be identical. The cesspool coccus at present liquefies gelatine and peptonizes milk somewhat more slowly than our coccus, but otherwise its cultural characters are indistinguishable from those of the latter, and morphologically the organisms are identical. Its pathogenic properties have not yet been tested.

A STUDY OF THE NEURONE THEORY.*

By MARTIN H. FISCHER.

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PLATES XXIII AND XXIV.

The opinion is now widely accepted, that the nerve cells throughout the central nervous system are independent structures. The cell processes, it is thought, invariably run out to end free, and one nerve cell has no further connection with any other than that of mere contact. This, in brief, constitutes the neurone theory which is so vigorously maintained by von Lenhossék, Golgi, Ramón y Cajal and others.

The claim for the independence of the nerve cells is based almost entirely upon the use of Golgi's silver impregnation method, and the embryological researches of His. The investigations of the latter may indeed show that all the dendrites of a nerve cell develop from the neurocyte, and that they do not anastomose with each other; but when in mature cells such anastomoses are demonstrated anatomically, the observations of His lose some of their significance. But notwithstanding the fact that Golgi's method demonstrates only individual elements, and these for the most part only imperfectly; and notwithstanding the fact that the pictures presented are so crowded with artefacts as to cause every statement concerning the histology of the cells to be made with the greatest reserve, still as a result of its use, the old observation of Gerlach (1), that the nerve cells anastomose with each other, has been discarded, and the more modern neurone theory substituted.

Held (2) does not believe in the neurone conception and maintains that anastomoses between nerve cells are demonstrable by Golgi's method. My own observations have led to a similar conclusion.

* A preliminary communication was read before the Chicago Pathological Society, April 10, 1899.

Even after obeying von Lenhossék's instructions to choose for investigation only single, isolated impregnated cells, anastomoses have been demonstrated, whose genuineness, to my mind, is not to be doubted. My observations are based upon a study of the cerebral cortex, basal ganglia, and motor spinal ganglion cells of the white rat and of the spinal cord of man.

In the cerebral cortex, the pyramidal cells are usually connected in a sort of end-to-end manner, the large dendrite of one cell passing upward and into the body of another cell directly above (Plate XXIII, Fig. 1) or above and to one side of it. This same condition has been described by Růžička, who, however, working with toluidin blue, employed a totally different technique. Thus two entirely different methods have given the same results. A few times I have found these pyramidal cells to be connected by transversely running dendrites, which join together cells lying in about the same plane in the cerebral cortex (Plate XXIII, Figs. 2 and 4). In the basal ganglia, the cells are connected in various ways, the usual arrangement consisting of short protoplasmic bridges which connect the two cell bodies (Plate XXIII, Fig. 5). The spinal ganglion cells furnish interesting pictures of anastomosing cells. For the most part two cells are found connected by a common dendrite; but three are not uncommon, and in one case an anastomosis between five cells was noted (Plate XXIII, Fig. 3).

Since, however, the upholders of the neurone theory will in no case admit an anastomosis which is demonstrated by Golgi's method (condemning such in every case as only apparent), we are driven to adopt another method to settle this dispute.

Dogiel, who has demonstrated anastomoses between the nerve cells of the retina, has employed Ehrlich's method of the intravenous injection of methylene-blue. Bethe (3) has used molybdic-acid toluidin blue, while Růžička (4) has worked with plain toluidin blue; both have found that the nerve cells not only come into contact with each other, but actually anastomose.

In my investigations of this subject, I have employed, besides Golgi's method, the following modification of Nissl's method:

(*a*) The tissue is hardened in a 10 to 20 per cent solution of formalin, cut with a freezing microtome, or dehydrated, embedded in celloidin or paraffin, and sectioned.

(*b*) The sections, which should not be too thin, are stained from one to twenty-four hours in a solution of Grübler's soap methylene-blue which has been diluted with an equal amount of water.

(*c*) The specimen is rinsed in water and dehydrated and differentiated in aniline-oil alcohol (1-20).

(*d*) Cleared in oil of cajeput and mounted in Canada balsam.

I consider this method superior to Ehrlich's intravenous injection of the dye for this purpose, as the pictures obtained are more intensely stained. Růžička's method is, I think, improved by the use of formalin instead of corrosive sublimate, as formalin acts as a powerful mordant for the dye, and this permits of a longer period of differentiation in alcohol, making the resulting pictures stand out more prominently.

The nerve cells in specimens colored by the method I have given are overstained. The cell processes are stained for long distances from the cell body, as are also the branchings and subbranchings. Furthermore, all neighboring structures are stained, so that the true relation of these to the neurones can now be studied to better advantage. The Nissl bodies are plainly visible in the cells and dendrites, so that the identity of these structures is never doubtful. The nucleus appears usually as a somewhat lighter blue spot in the centre of the cell body.

It is now an easy matter to see that the nerve cells are not separate individuals, but frequently anastomose with each other. The relation of two cells is not always one of mere contact only, but one of actual connection between the protoplasm of one cell and that of the other.

The two cell bodies are in these cases connected with each other by a common dendrite (Plate XXIV, Figs. 6, 7, 8), which is in every way similar to that ordinarily observed. The process arises from the one cell by its usual, broad, wedge-shaped base, and its connection with the other cell is marked by a similar base. The Nissl bodies can be traced throughout the entire length of the dendron. The connecting dendrite is usually a short, protoplasmic bridge (Plate

XXIV, Fig. 6), but quite often extends to a greater length (Plate XXIV, Figs. 7 and 8). There is never any doubt as to whether the anastomosis is a true or only an apparent one. The dendrites are not opaque as in Golgi's method, but translucent, so that in every case it can be easily seen whether the protoplasmic processes really connect with each other or only touch each other. Thus far I have found anastomoses only between cells of the same group, and never between cells of different groups, an observation which is in harmony with that of Růžička.

Unfortunately the methylene-blue staining does not lend itself well to photomicrography, so that I have been forced to use drawings to illustrate my observations.

Sala (5), working with Golgi's method, has put forth the claim that the dendrites embed themselves in the blood-vessels. His position has been substantiated by Růžička, but is severely criticised by von Lenhossék. That some of the dendrites do actually embed themselves in the walls of the capillaries, is, to me, an established fact. The tip of the cell process, before it connects with the wall of the capillary, widens out into a wedge-shaped end (Plate XXIV, Fig. 9). The occurrence of this condition leads me to believe that the dendrites have, besides the generally accepted nervous, also a nutritive function.

As the result of this study, I would point out the following:

First. The neurone theory, in so far as it claims the absolute independence of the neurones is an untenable one, as anastomoses between them have been found.

Second. The dendrites, which are generally believed to have but nervous function, may have also nutritive function, if such an inference is permissible from the existing anatomical relations, which show some of the dendrites embedding themselves in the walls of the capillaries.

DESCRIPTION OF PLATES XXIII AND XXIV.

PLATE XXIII.

Fig. 1. Photograph showing anastomoses between the pyramidal cells of the cerebral cortex of white rat. Method of Golgi.

Fig. 2. Anastomosis between the nerve cells of the cerebral cortex of white rat. The dendrite seen running upward and to the right from the

upper neurocyte is the continuation of the delicate cell process which is seen to the right of the heavy common dendrite. Golgi method.

Fig. 3. Anastomoses between five motor ganglion cells of the dorsal spinal cord of the white rat. Golgi method.

Fig. 4. The bodies of two pyramidal cells of the cortex connected by a common dendrite. Brain of white rat. Golgi method.

Fig. 5. Two nerve cells of basal ganglia connected by common dendrite. Golgi's method.

PLATE XXIV.

Fig. 6. Two motor spinal ganglion cells connected by short protoplasmic process. Methylene-blue staining.

Figs. 7 and 8. Motor spinal ganglion cells from cervical region of human spinal cord. Methylene-blue staining.

Fig. 9. A dendrite embedding itself in the wall of a capillary. Human spinal cord, anterior horn cell of cervical region. Methylene-blue staining.

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5. Sala, L.—Zur feineren Anatomie des grossen Seepferdenfusses, *Zeitschr. f. wiss. Zool.*, Bd. III., Taf. V., Fig. 6.

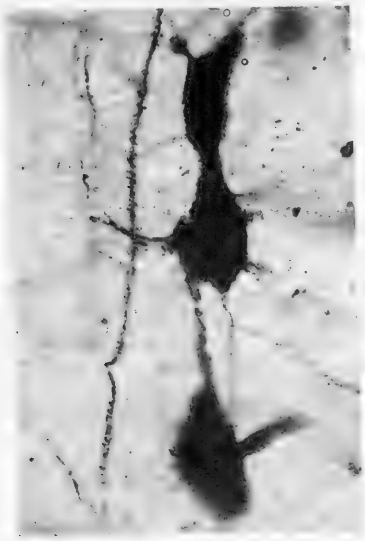


FIG. 1.

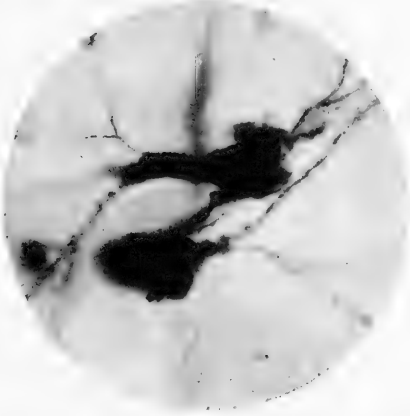
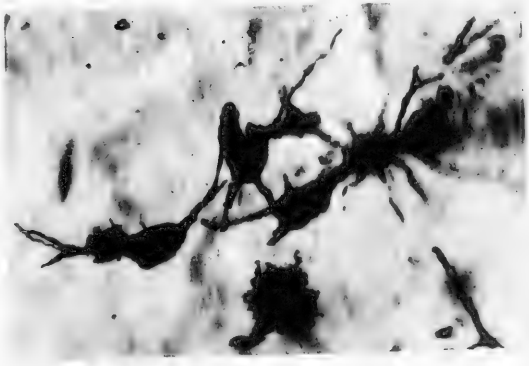


FIG. 2.



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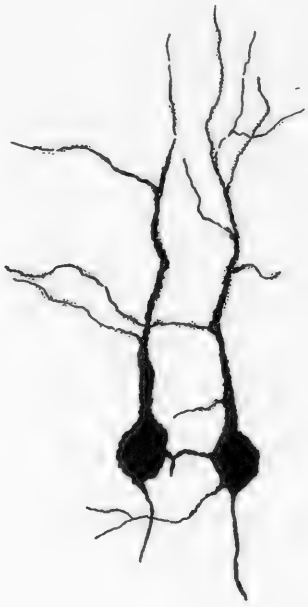

FIG. 4.

FIG. 5.

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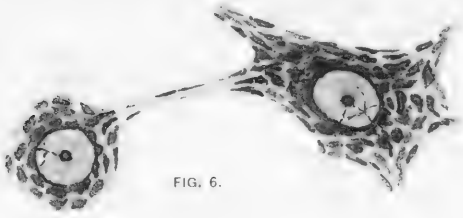

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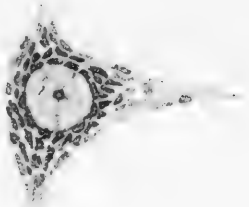

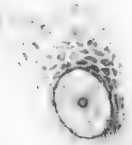

FIG. 7.

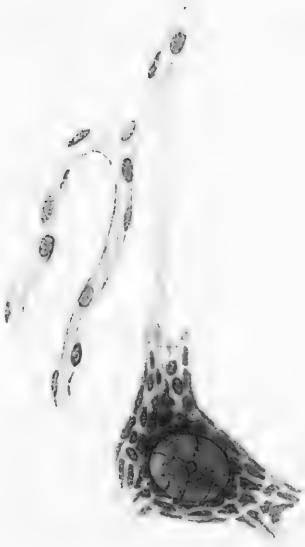

FIG. 9.

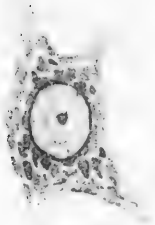

FIG. 8.

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A CONTRIBUTION TO THE SUBJECTS OF CHRONIC INTERSTITIAL NEPHRITIS AND ARTERITIS IN THE YOUNG, AND FAMILY NEPHRITIS; WITH A NOTE ON CALCIFICATION IN THE LIVER.

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Plate XXV.

Chronic nephritis in children and adolescents is a condition which has been much overlooked. Especially is this true of the chronic interstitial type (contracted kidney). Dickinson was of the opinion that the latter condition was not infrequent in children. Heubner (1), who has lately written a monograph upon this subject, from which many of these introductory remarks are cited, states that of 251 cases of chronic nephritis which he observed in Leipsic, 214 occurred in adults and 37 in children. Later, in Berlin, he saw 28 cases in children, mostly after scarlet fever. Of these 65 cases, there were 3 of parenchymatous nephritis, 4 of contracted kidney and 5 of chronic hæmorrhagic nephritis (Wagner). Of 33 cases of chronic interstitial nephritis observed by Bartels (2), one occurred at the age of 18, one at 19, and two at 20. Of Dickinson's (3) 308 cases, one occurred between 11 and 20 years, and 24 between 21 and 30 years.

From these figures it is seen that under the age of 20, chronic interstitial nephritis is uncommon, but not so rare as the omission of reference to it in many text-books on children's diseases would indicate. The condition is even more frequent than these figures would lead us to infer, as many of the cases are overlooked and treated for anæmia for a long time. This is especially true of the subacute cases of productive nephritis described by Delafield (4).

Of the interstitial cases, which alone interest us here, Heubner states that there are but thirty on record in which post-mortem examinations were made. Besides these, there are numerous other instances reported in which the patients were not observed until the fatal termination, or where no autopsy was performed. To these cases, Heubner adds four from his

own experience; three in males, aged respectively 14, 9 and 24, and one in a female aged 11. Of these four cases, however, one was probably of amyloid disease. In none of these cases is there any post-mortem examination recorded. In all the cases which Heubner cites the disease was very insidious, but was easily discovered upon properly examining the patients. As a rule the disease lasted for many years. It can, however, be rapid in its course, as in Crooke's (5) case, where, at the autopsy on a boy nine years old, who died on the 68th day after the onset of scarlet fever with diphtheria, the kidneys were found to be distinctly granular. Delafield (4) has drawn especial attention to these rapid cases (acute productive nephritis).

According to Heubner, there are seven cases in the literature in which there is no history of a previous acute attack, so that they can be looked upon as instances of primarily contracted kidneys. These are the cases of Barlow (6), Bull (7), Filatoff (8), Morell-Lavalée (9), Oppenheim (10), and Förster (two cases) (11). The case reported by Morell-Lavalée is of a special type, as the condition was complicated by a cirrhotic liver. In the other cases, which died at the ages of 5, 6, 10, 13, and 14 years respectively, it is noted that the children were always weak, of capricious appetite, pale, and had weak resisting powers.

Accidentally, or at the occurrence of a slight œdema, the albumin in the urine was discovered. It is, of course, possible, as Heubner states, that these children had had an attack of acute nephritis in earliest infancy or childhood, so that the question of the occurrence of a primarily contracted kidney in children must remain an open one. Of the four cases which Heubner has seen, one is probably a case of amyloid kidney as stated above, and two are probably secondary cases. Only one is possibly a primary case. This is that of a boy aged 9 years, who presented typical symptoms of chronic interstitial nephritis, but of whose history only a part is known.

It may be of interest to give details of some of the reported cases:

Barlow's case was that of a girl aged 6 years. She suffered from headache, pallor and vomiting, and died with uræmic symptoms. At the autopsy there was found a very marked chronic interstitial nephritis. The right kidney weighed 13 grammes and measured 5x4x0.5 cm. The left weighed 21 grammes and measured 6x3¾x1¼ cm. The arteries were thickened.

Bull's case was that of a girl thirteen years old. The left kidney weighed 20 grammes, was 5 cm. long and 3 cm. wide. The right measured 7½x4½ cm.

Filatoff's case was of a girl aged 12, who was always weak and passed

very much urine. In her 11th year, she had an apoplectic attack with left-sided hemiplegia. Later she had headache and vomiting and began to lose her eyesight. An albuminuric retinitis was found. Before her death she became slightly œdematous and had a severe nasal hæmorrhage. The urine was clear, 1500 cc. daily, and slightly albuminous. The arteries were tense and hard, the apex beat was exaggerated and situated below and to the left of the normal position. The autopsy showed granular kidneys, hypertrophy of the left ventricle, endarteritis of the aorta and the vessels at the base of the brain, and an old hæmorrhagic extravasation in the right corpus striatum.

In Lavalée's case, a child 5½ years old suffered from ascites with fever At the post-mortem examination there was found cirrhosis of the liver, spleen and kidneys.

We have not been able to consult Oppenheim's article.

Förster's two cases occurred in the same family. The boy died at the age of 9½ years, after an illness of 5 years' duration. The autopsy showed typical contracted kidneys. The girl died at 8¼ years, having been ill 3½ years. Several weeks before her death she developed a hæmorrhagic diathesis, having several severe hæmorrhages from the mucous membrane of the mouth. An autopsy was not performed. Both cases during life resembled cases of diabetes insipidus.

To these seven cases gathered by Heubner, making eight with his own case, we may add a few others which we have found. Westphal (12) reports the case of a man aged 24 years in which there was found an atrophic kidney on one side and a contracted kidney on the other. There was no history of any previous illness and the patient succumbed after having been sick a very short time. It is of interest to note that Curschmann had diagnosed the existence of a contracted kidney with the probability of one kidney not functioning. Baginsky (13), in his textbook, mentions a case in a girl aged 4, which stimulated diabetes insipidus. There is no autopsy reported. Ashby and Wright (16), mention two cases which seem to belong in this group. They both occurred in girls, one 11½ and the other 10½ years. v. Buhl reported a case in a child of one and one-half years (cited by Eichhorst (28)).

It is a remarkable fact that even congenital cases have been described. Baginsky (13) mentions two such occurrences in his experience but gives no details. Weigert (14) describes briefly the following case: At the autopsy performed on a child six weeks old, who had suffered from marked cyanosis, there was found an atresia of the pulmonary artery, an open septum and hypertrophy of the right ventricle. The kidneys measured 1.7x1x0.5 cm. and 2x1.1x0.4 cm. The cortex was narrow and

the substance pale and firm. There were small cysts on the surface. Microscopically, there were found fairly normal parts with fatty degeneration of the epithelium and areas of marked chronic interstitial inflammation. The epithelium of the Malpighian tufts still consisted of large cells, and, like the cortical cells, showed fatty degeneration. Hellendall (14*a*) has reported two instances of contracted kidney, probably of congenital origin, in infants, the one six months and the other two years old.

The following case, which was observed at the Mount Sinai Hospital, adds another to this list of cases, and it is so interesting from many other points of view that it is considered worthy of publication.

Ida W., school-girl, aged 14 years. Admitted January 13, 1898. *Family history* is of special interest and will be given in detail subsequently (p. 554).

Previous history: The girl has always been weak and under-sized. She generally stayed at home because running around on the street made her short of breath. She has never menstruated. One year ago, her face and feet were swollen during the whole winter. She always felt better during the summer. For some time past she has had a cough with expectoration. When six months old she suffered from an attack of gastro-enteritis of a few days duration. She has had no other known acute illness.

Present history: One week ago, she became very much frightened at a fire in the neighborhood at 2 P. M. She was apparently well until 11 P. M., at which time she went to sleep. The next morning it was noticed that the left side of the body was paralyzed. She complained then and still does of headache. She had fever for the first time three days ago, and two days ago she coughed up a small quantity of blood. Urination and defecation are stated to be normal. Her intelligence and memory have not been impaired. The paralysis has improved somewhat; she has twitchings of the entire left side of the body.

Physical examination: Under-sized girl, poorly nourished, with a dirty, anæmic color. The tongue is moist and coated. There are no defects in the teeth and there is no lead-line present. There are no signs of any corneal inflammation, and no changes in the ears. She has a left-sided hemiplegia with some rigidity. Sensation is unimpaired in the upper but diminished in the lower extremities. *Lungs*, negative. *Heart*, dulness extends from 1 cm. to the right of the sternum to 5 cm. to the left of the mammillary line. The upper border is at the third

rib. The apex beat is in the fifth space in the axillary line. It is forcible and fairly localized. The heart's action is tumultuous and forcible. There is a systolic murmur over the mitral area, transmitted a short distance to the left. Over the aortic orifice there is heard a systolic murmur transmitted up and down the sternum and to the vessels of the neck. The second aortic sound is accentuated and reduplicated. The radial *pulse* is tense, the artery is very much thickened and slightly tortuous. The *liver* dulness extends from the fourth space to the free border of the ribs. *Spleen*, negative. *Abdomen*, negative. The right pupil is slightly larger than the left.

Subsequent history: January 12, 1898. Pulse 120. Respiration 32, temperature 101.8° F.

January 13. Urine neutral, 1010, clear, and contains albumin—1.4 grammes to the litre (Esbach); urea 6½ grains to the ounce; hyaline and granular casts and a few pus cells are present. The quantity of urine could not be measured because the urination was involuntary. She has some cough and complains of pain in her stomach.

January 14. Temperature normal; labial herpes; patient somewhat soporose.

January 15. Urine contains albumin, red and white corpuscles, but no casts.

January 17. The movements from the bowels contain some blood.

January 19. Urine again shows hyaline and granular casts.

January 20. Mental condition has improved. Paralysis unchanged. Pain in abdomen less marked.

January 30. Marked restlessness.

February 3. Temperature elevated to 103°. She complains of pain in the left hypochondriac region. By percussion the spleen is found to be enlarged. It can be felt and is tender.

February 5. Temperature 104.6°. Has severe coughing spells. Over the first four interspaces on the right side anteriorly and posteriorly are numerous crepitant râles, increased voice and breathing and a dull percussion note.

February 6. Temperature 102°.

February 7. Over right lower lobe behind there is bronchial voice and breathing. Severe abdominal pain. Temperature 100°.

February 8 to 10. Patient's condition worse. She lies in a semi-comatose condition and complains of severe abdominal pain. The hemiplegia has remained unchanged. The physical signs on the right side have partially cleared up. Electrical examination shows an almost com-

plete loss of reaction to the faradic current in both the left upper and lower extremities.

February 15. Ulcers found on the upper and lower gums with necrotic, easily bleeding bases.

February 18. Over the upper lobe of the left lung is an area of dulness, increased voice and breathing, crepitant râles. Hæmoptysis.

February 23. Necrotic areas in the mouth have increased in size and number. Ophthalmoscopic examination shows retinal hæmorrhages.

February 25. Continued hæmoptysis with signs of consolidation of a large area in the left lower lobe posteriorly.

February 26. Temperature suddenly drops to 96.4°. The patient had very severe pain in the abdomen and passed a large amount of blood with the stool. Pulmonary œdema.

February 27. Temperature still remains subnormal. Marked tympanites. Subcrepitant and crepitant râles heard in front and behind below the second rib down to the base on the right side. Râles heard yesterday have almost entirely disappeared. Urine contains albumin, 16 grammes to the litre. Urea 4 grains to the ounce. Temperature subnormal.

March 3. Very marked pericardial friction sounds heard even at a distance from the patient.

March 5. Crepitant râles over the upper part of the left chest with bronchial voice and breathing. Temperature 94° to 96°.

March 6. Return of the pulmonary œdema. Exitus.

Clinical diagnosis: Chronic interstitial nephritis. General arteritis. Atheroma of aorta and aortic valve. Cardiac hypertrophy. Cerebral hæmorrhage. Hæmorrhages into the lung and spleen. Hæmorrhages in the area of distribution of the superior mesenteric artery. Fibrinous pericarditis.

Post-mortem examination: Five hours after death. Body very much emaciated. Slight general œdema, more marked on the left side of the body, with marked œdema of the feet.

Lungs: Slightly adherent at the apices. Moderate pulmonary œdema. Both lungs are very firm. In the right upper lobe there is a very dense non-crepitating area which is infiltrated with blood. In the right lower lobe there is a similar smaller triangular area. In the left upper lobe, are several patches of consolidation and one large hæmorrhagic area. In the left lower lobe, there is a large recent hæmorrhage. There is marked subpleural emphysema of both lungs.

Heart: On the anterior aspect there is very much fresh fibrin; the left ventricle shows marked hypertrophy, the thickest part of the wall

measuring 3½ cm. and the thinnest 2 cm. The aortic valves show moderate atheroma. The flaps of the mitral valve are slightly thickened. The wall of the right ventricle is somewhat hypertrophied. The aorta shows patches of atheroma especially around the openings of the coronary arteries. The coronary arteries are markedly atheromatous. On the endocardium between the aortic and mitral valves, and on one cusp of the mitral, are patches of marked atheroma. The cardiac muscle is pale but firm.

Spleen weighs 80 grammes; measures 5x8½ cm. Malpighian bodies are very distinct. Almost the entire spleen is taken up by a large hæmorrhage.

Kidneys, small, red. The right weighs 59 grammes and measures 9x4x3 cm. The left weighs 34½ grammes and measures 6¼x3½x2½ cm. The capsules are very adherent; the surface irregularly granular. The kidney substance is firm; the cortex is very narrow. The pelves of both kidneys are markedly enlarged, and surrounded by fat. The markings are very indistinct. On the surface of the kidney are enlarged veins, and some of the glomeruli can be indistinctly seen as white dots. The left kidney is more markedly affected than the right, the parenchyma being only 0.5 cm. wide.

Stomach: Catarrhal inflammation. A few hæmorrhagic erosions near pylorus.

Intestines: There is a large hæmorrhage in the mesentery. No emboli nor thrombi can be found in the mesenteric arteries or veins or their branches. The superior mesenteric artery is markedly atheromatous. The small intestine shows catarrhal inflammation; the contents are mucoid and bloody. The large intestine shows catarrhal inflammation with marked injection of the walls.

Pancreas is indurated.

Liver somewhat enlarged, is fairly firm, shows chronic congestion and some increase in the connective tissue. In the right lobe on the anterior and inferior surfaces are triangular areas reaching to the surface in which there is a firm deposit in and along the vessels looking as if the vessels were firmly thrombosed.

The brain could not be examined.

Microscopical examination: Lungs show the changes characteristic of brown induration with a fair amount of increase in the interstitial connective tissue. The lower lobe shows areas where the air-vesicles are filled with epithelial and pus-cells and numerous red blood-corpuscles. The right upper lobe shows the same condition with a larger and more recent hæmorrhage. The left upper lobe contains a large area of

croupous pneumonia. The left lower lobe shows marked recent infiltration with red blood-corpuscles.

Liver: The capsule is thickened. The capillaries are dilated and there is a little increase in the interstitial connective tissue. The liver cells show marked degeneration and pigmentation. There are small hæmorrhages under the capsule. The walls of the hepatic veins are thickened and the veins themselves are dilated. The arteries show marked obliterating endarteritis. Scattered throughout the parts of the liver which showed such a peculiar condition macroscopically, are irregular areas (Plate XXV, Fig. 1) which stain very darkly with Delafield's hæmatoxylin and look necrotic. These areas seem to follow the branches of the hepatic artery, surrounding and sometimes obliterating the lobules. On the addition of dilute hydrochloric acid to the unstained specimen, these areas become much paler without the evolution of gas bubbles, and when thoroughly washed out and then stained with the same solution, they appear more homogeneous and stain with about the same intensity as the surrounding parts of the liver. They are, therefore, areas which have become impregnated with lime salts. The absence of any evolution of gas bubbles on the addition of the acid shows that they consist of calcium phosphate.

Spleen shows the changes of acute inflammation. The capsule is thickened and there is an increase in the interstitial connective tissue. The pulp is infiltrated with red blood-corpuscles. The arteries are the seat of a marked obliterating endarteritis.

Kidneys (Plate XXV, Figs. 2 and 4): The right kidney shows a marked chronic interstitial nephritis. In some areas the renal parenchyma is entirely replaced by fibrous tissue. Many of the glomeruli are converted into fibrous balls. Some of the tubules are dilated, others are atrophied; some contain casts, others granular material or blood. The epithelium shows marked degeneration. There are small scattered hæmorrhages present and signs of chronic congestion. The arteries show marked obliterating endarteritis (Plate XXV, Figs. 2 and 3), many of them being entirely closed. Scattered throughout the kidney are very small areas similar to those which have been described as occurring in the liver. The left kidney shows the same changes as the right, and in even more marked degree.

Pancreas contains areas of infiltration with round cells between the lobules, and in some places in the centre of the lobules.

Coronary arteries: There is extreme thickening of the intima, fibrous metamorphosis of the media and extensive calcific deposits and necroses in the intima (Plate XXV, Fig. 5). One of the smaller peripheral

arteries shows, microscopically, a thickening of all the walls; the intima contains calcified and necrotic areas, and there are broken-down thrombi attached to it. The media is almost entirely replaced by connective tissue. There are small hæmorrhages in the adventitia. *The superior mesenteric artery* likewise shows irregular thickening of the intima and of the media with small calcific areas in the intima.

Anatomical diagnosis: Pulmonary œdema; brown induration of lungs; hæmorrhages into lungs, spleen and mesentery; lobular pneumonia; cardiac hypertrophy; general chronic arteritis; chronic congestion of the liver, with calcific deposits; acute splenic tumor; catarrhal inflammation of the gastro-intestinal tract; chronic interstitial nephritis (small red kidney).

Remarks on the results of the post-mortem examination.—The kidneys from this case are among the smallest that have been recorded in the chronic nephritis of early life. In a few of the other recorded cases, the size of the kidneys has been as follows: Handford (15), 12-year-old girl, right kidney, 60 grammes; $8\frac{1}{2}$x$4\frac{1}{2}$x$2\frac{3}{4}$ cm. Left kidney, 15 grammes; 5x4x2 cm. In Ashby and Wright's (16) cases, from the one case of a girl $11\frac{1}{2}$ years old the right kidney weighed 75 grammes, the left kidney, 22 grammes. The kidneys from the other case weighed together 45 grammes and each measured 5 cm. in length. In one of Kidd's cases (17) one kidney weighed 60 grammes and the other 30 grammes.

The change in the liver, which is reproduced in Plate XXV, Fig. 1, is certainly very striking. In places it made the impression that the process began in the walls of the arteries and that then the parts supplied became degenerated and infiltrated with lime salt. The occurrence of such calcification in the arteries, beginning in the intima, was demonstrated in the kidneys and is illustrated in Plate XXV, Figs. 2 and 3.

The presence of calcified areas in the liver is extremely rare. Babes mentions an instance in an article on the technique of staining with safranin.* The liver described by him was obtained from a patient who suffered from tuberculosis of the femur and the hip-joint.

* Virchow's *Archiv*, 1886, cv, p. 511.

None of the other organs showed similar changes. The liver was large, grayish-brown in color, and presented grayish-yellow patches corresponding to the central veins, so that it looked like a nutmeg liver of a peculiar character. It felt sandy, and cutting it caused a grating sound. The deposit proved to be composed of lime salts in and around the central veins and their branches and in the surrounding cells. Around these areas the cells had lost their nuclei and their contours were obliterated. Babes considered the condition to be due to lime resorption from the bone disease and its later deposition in the liver. Virchow thought likewise, but the exact manner of its occurrence could not be determined.

Similar, but smaller and fewer areas of calcareous impregnation, were found in the kidneys and in the walls of many of the vessels. Figure 5 (Plate XXV) shows the deposits in the walls of the coronary artery. It also shows the other changes noted in the report of the microscopical examination. In Fig. 4 (Plate XXV) are shown the changes in the kidney, the part represented, however, not being that most markedly affected. The large number of vessels almost or entirely closed is very striking. As stated above, an examination of the brain was not permitted, but it is probable that the hemiplegia which marked the onset of the symptoms was due to a right-sided hæmorrhage.

For the sake of completeness we may note here that a blood culture was made 14 hours before death. A pure culture of Staphylococcus albus was obtained, and after death the same organism was cultivated from the heart's blood. We lay no stress upon this observation, as the agony was very prolonged and the bacteræmia may have been due to an agonal invasion.

General Remarks.—(1) As to the diagnosis of the main conditions, only a few words are necessary. At first sight the case might have been considered as primarily cardiac in nature with secondary renal changes of the chronic interstitial type. The marked hypertrophy of the heart, however, with the distinctly accentuated second sound caused us to suspect a primary affection of the kidneys. The finding of the diseased radial vessels supported this view. It must be remem-

bered, however, that a similar clinical picture can be produced by disease of the vessels without involvement of the kidneys. This fact, which has not received proper recognition, is strikingly illustrated by a case recorded by Hawkins.* As this case resembles ours to a great extent, we present it in detail:

A girl, aged 11 years, in the summer of 1891, had a week's illness, probably attributable to infarctions of the lung. In January, 1892, similar symptoms again appeared, the pain being first on the left side and later on the right. The cough, with blood-stained sputum, persisted up to the end of the illness. February 7, the legs became œdematous and later the face. The urine showed a trace of albumin, no casts, and was diminished in quantity. February 25, the urine was bloody and contained a little albumin, and the patient vomited. The child died after three weeks from what seemed to be uræmic poisoning, the case being considered one of acute nephritis. There was no syphilitic history. At the autopsy all the arteries of both the aortic and pulmonary systems were found to be diseased. There was consolidation with infarctions of portions of both lungs. Both renal arteries were occluded by thrombi. There were small clots in each of the lateral lobes of the cerebellum. The left ventricle was dilated and hypertrophied. For a distance of three inches above the bifurcation the aorta was so narrow as scarcely to admit a bullet-probe. The arterial wall measured only one-half inch in breadth when opened. The kidneys microscopically showed no signs of nephritis. The arteries, macroscopically, showed gray translucent spots and patches, smooth or corrugated. Microscopically, these were composed of indistinct fibrocellular tissue in the intima. In the media and adventitia were collections of leucocytes, especially around the vessels. The new tissue was not the seat of fatty or degenerative change, with the exception of some deeply-stained patches of amorphous material (lime?) on the surface.

(2) The hæmorrhages in our case caused prominent symptoms during life. Those in the lungs were easily made out. The intrasplenic hæmorrhage was diagnosed from the acute enlargement and tenderness of the spleen and the rise in temperature. The extravasation in the mesentery was indicated by excessive abdominal pain, bloody stools, and tympanites; and the sudden occurrence of sub-

* *Transactions of the London Pathological Society*, 1892, p. 46, cited in part by Delafield (4).

normal temperature indicated a large hæmorrhage. The question whether the hæmorrhages were due to thromboses, embolisms or ruptures of smaller or larger vessels had to be left open as any one of these conditions was possible. The marked bleeding from the gums, however, indicated that it was not necessary to assume the existence of embolism or thrombosis and that the hæmorrhages might be explained by the existence of a hæmorrhagic diathesis. Such a diathesis in similar cases has been noted by Filatoff (8) and Förster (11).

(3) The arterial changes present were very advanced. The question naturally arises whether these or the renal changes were primary. This is frequently a very difficult problem. From the examination of two other members of the same family, we are inclined to believe that the lesion in the kidney was primary, as these two persons have long-standing chronic nephritis with but little evident arterial change. Of course, it is possible that their peripheral arteries may not show arteritis, while this may exist elsewhere, and, therefore, a positive opinion is not warranted.

Throughout the records, there are occasional references to cases of chronic arteritis in children, and it has been noted that there exists in certain families a tendency to the development of arterial changes early in life. The most common occurrences have been aneurisms of the cardiac valves and dilatation of the aorta (Baginsky). Jacobi, (18), in reporting a case of aneurism of the abdominal aorta in a child, refers to twenty-eight other recorded cases of aneurism in early life. Since then at least two more cases have been described. These cases need classification, however, as some of them were not due to primary arterial changes but were mycotic in origin (Eppinger). Among the striking instances of advanced arterial disease reported in childhood, are the following:

Fenomenoff has recently reported a congenital aneurism of the abdominal aorta which obstructed delivery (Eichhorst). Oppe (19) describes an aneurism of the basilar artery in a boy seven years old. Sanné (20) reports the case of a child of two years with narrowing of the aorta due to chronic aortitis. Pendin (21) describes in a girl of 12 an aneurism of traumatic origin. Andral (22) saw calcific plates in the aorta of

a girl 5 years old. Moutard-Martin (23) saw atheroma of the arch in a boy aged two years. Hodgson (24) records calcification of the temporal artery in a girl of five, and Hoffnung (25) describes an aneurism of the pulmonary artery in a girl of ten months. Generalized arterial disease in connection with chronic interstitial nephritis without any syphilitic history has been described by Filatoff (8) and Barlow (6).

The changes found in our case were as advanced as in well-marked arteriosclerosis of adult life. While the character of the lesions suggested syphilis, a very thorough examination of the whole family failed to elicit anything corroborative of such a view.

(4) The absence of any usual etiological factor drew our attention to the possibility of the case being an instance of so-called "family nephritis." Senator's recent volume on the diseases of the kidneys, in Nothnagel's Handbuch, cites several instances of nephritis occurring in several members of the same family.

Dickinson (26) reports a family in which in the first generation two sisters had albuminuria for many years and died at the ages of 48 and 49. Of the four children of a brother, one son had albuminuria for 14 years and died at the age of 26, and one daughter died at 34, after having been sick sixteen years. Two other daughters had no albuminuria and lived to the ages of 38 and 40. Of six children in the third generation, five had albuminuria, one daughter having it, from the age of nine months, for twenty years, and one son of 20 for an unknown length of time. Another son of 15 had it for two years and one daughter of five years was albuminuric since the age of six months.

Tyson (27) mentions the instance of a man of 30 with contracted kidneys whose father and mother died of the disease, and one brother at the age of 37. Two children of the brother had chronic nephritis at the ages of four and seven respectively. One brother died at 29 from convulsions. Two older brothers and one sister, aged 23, 32 and 36 respectively, had the disease for five or six years. One cousin on the mother's side and numerous relatives in earlier generations died of chronic nephritis.

Eichhorst (28) gives the history of an artist's family who were sufferers from the disease. The grandmother (who had no gout) died of uræmia. The mother and a daughter 24 years old suffered from chronic interstitial nephritis, the former for fifteen years. Two sons died of

uræmia. A daughter, aged 22, showed signs of the disease at the time of the report.

Kidd (17) reports the case of a woman of 60 who died of nephritis of long standing, as did also her brothers. Of 12 children, 7 died with the same disease, and two had it at the time of his report. In two of the fatal cases a post-mortem examination was made and contracted kidneys were found. Possibly Förster's cases belong in this category.

Samelsohn (29) observed chronic nephritis in two brothers, and noted its probable existence in two sisters and a son in the same family.

Pel (30), in a recent article, mentions several families in which he has noted the tendency to the development of chronic nephritis, and reports one instance of family nephritis not less striking than that of Dickinson. The disease affected 18 members (9 males and 9 females) of the family in three generations. Of 60 children of the fourth generation, none at the time of Pel's report was affected, but as, in contrast with the observations cited above, the disease in this series appeared later in life, their subsequent fate must remain for the present in doubt. Pel notes that in this family the tendency was for the sons to inherit from the father and the daughters from the mother—an exception to the Darwinian rule of heredity.

An examination into the family history of our case and a personal investigation of the members of the family, which was possible only after repeated efforts, revealed the following interesting facts:

The father died at the age of 42 of lobar pneumonia. His previous history was negative so far as can be determined. The mother is 45 years old and in good health, presenting no signs of renal or arterial disease. Of the 12 children, six died very young of causes which are unknown. The living children are as follows:

1. A girl of eight, who seems to be in good health and whose urine and heart show no changes.

2. A boy, aged 13, who is also in good health and in whom the physical and urinary examinations reveal nothing abnormal.

3. A girl of fourteen, who complains of severe headache. She is very well developed and her heart and arteries show no deviation from the normal. The urine has a specific gravity of 1016 and contains no albumin nor casts.

4. A girl, aged 19, who has been ailing for at least six years (probably over 10 years). She has no history of any acute disease. Menstruation be-

gan at 12 and has always been irregular and profuse. She complains of very great thirst and has to arise several times at night to drink water. She passes very much urine. The appetite is poor. She has no headache and no vomiting. She has occasional cough and palpitation, but complains only of her excessive thirst. An examination on November 5, 1898, showed the following: There is marked anæmia. The pulse is of high tension and the walls of the radials are somewhat thickened. The sphygmographic tracing shows marked elastic vibrations and only a slight dicrotic notch. The apex beat is in the fifth space just to the left of the mammillary line and is very forcible. The urine is very light in color and has a specific gravity of 1001½. There is albumin present, 0.14 per cent (Esbach), no sugar. The microscopical examination shows only epithelium and a few leucocytes. When seen two weeks later, there was marked œdema of the eyelids and some headache, which disappeared after a few days. At the date of writing, March 1, 1899, she is in good health, except for her inordinate thirst. She is evidently suffering from a very advanced chronic interstitial nephritis, which is running a slow and quite latent course.

5. The oldest son, aged 24, gives no history of any acute disease in childhood. He has always been anæmic. Six years ago, he suffered all winter from a severe cough and had general œdema which began in the face. He has felt perfectly well since then and, therefore, for a long time opposed any personal examination. On February 19, 1899, the following facts were made out. There is a moderate anæmia. The apex beat is in the sixth space, one-half inch to the left of the mamillary line, and is very forcible. There is a booming first sound and an accentuated second sound at the apex. At the aortic orifice, there is a slight systolic murmur and an accentuated second. The pulse is 86, the radial artery is slightly thickened, the pulse shows moderate tension. The urine is clear, 1012; contains no albumin and no casts; urea 9 grains to the ounce. Although he has no symptoms at present, and the urine shows nothing abnormal, there can be little doubt from his history and physical examination, that he has a chronic interstitial nephritis and belongs to that unusual class of cases in which for a time at least the urine shows no abnormalities. We have been permitted thus far to examine only one specimen of the urine, and it is quite possible that when we next examine it we may find albumin.

It is evident that we have to deal with a family in which there is a tendency to the development of chronic interstitial nephritis, this having occurred in the three eldest children.

The main points of interest in the case herewith presented are as follows:

1. The occurrence of a very advanced primary chronic interstitial nephritis at the age of fourteen years.

2. Its presence in other members of the same family.

3. The extensive and marked arterial changes present.

4. The hæmorrhagic diathesis and especially the occurrence of a large hæmorrhage in the mesentery.

5. The occurrence of calcific deposits in the liver.

6. The case draws our attention again to the latency of some of these cases of chronic nephritis in children. There is no doubt that some of them have been regarded as instances of diabetes insipidus. The necessity of a careful and continued observation of the heart and vessels in such cases is apparent. Others are treated for a long time for anæmia without its cause being discovered. Still others do not present themselves for treatment until the fatal termination is close at hand, the patients having had no marked symptoms. The fact that chronic nephritis may run so latent a course and may occur at any age should lead us to pay as much attention to the examination of the urine of children as of adults.

We are indebted to Dr. F. S. Mandlebaum, the pathologist of Mount Sinai Hospital, for much valuable assistance in connection with the histological part of this investigation.

DESCRIPTION OF PLATE XXV.

Fig. 1.—Section of liver showing areas of calcification. Low magnification.

Fig. 2.—Section of kidney showing obliterating endarteritis, calcified vessel, and chronic interstitial nephritis. Low power.

Fig. 3. Section of calcified artery seen in Fig. 2, with higher magnification.

Fig. 4.—Section of kidney showing the lesions of advanced chronic interstitial nephritis.

Fig. 5.—Section of wall of calcified coronary artery of the heart.

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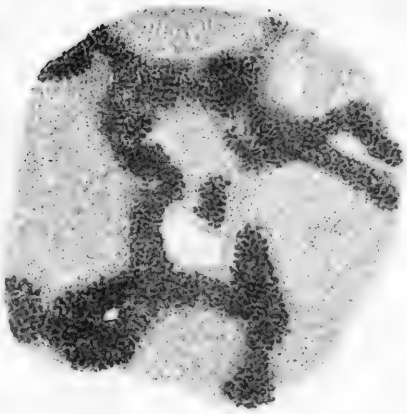

FIG. 1.

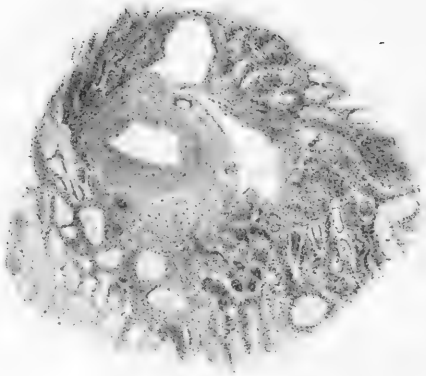

FIG. 2.

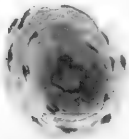

FIG. 3.

FIG. 4.

FIG. 5.

LYMPHOMA, A BENIGN TUMOR REPRESENTING A LYMPH GLAND IN STRUCTURE.

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Plates XXVI and XXVII.

There is little uniformity of opinion among authorities to satisfy the critical student concerning the word "lymphoma" and the conception conveyed by it. In conformity with oncological nomenclature, the term is, in a strict sense, applicable only to new growths made up of lymphoid tissue, but in the course of time it has come to include a variety of pathological conditions.

Kaufmann (1) and Orth (2) classify lymphomata into leukæmic, aleukæmic and malignant. Klebs (3), following Virchow, Birch-Hirschfeld (4) and Ziegler (5), emphasize the fact that in many instances inflammatory processes have monopolized the use of the term. Langhans (6) many years ago stated that "in pseudo-leukæmia there are two forms of lymphomata;" and Warren (7), in enumerating the conditions denoted by the word "lymphoma," mentions "simple hypertrophy of the lymphatic glands, due to some of the above causes (tuberculosis, syphilis and other infective diseases), to which the term lymphoma has been applied." Warren advocates dropping the term lymphoma, since "those cases which were supposed to occupy an independent position under the name of lymphoma or lymphadenoma can now be classified under some one of the other headings." The objection to this by Birch-Hirschfeld is that if the term lymphoma is limited to the infectious enlargements of lymph glands to which it has so far been mainly applied, there remains nothing by which to designate a benign lymphomatous tumor.

Most unsatisfactory is the definition of Stengel (8), who defines lymphoma as "a more or less malignant form of new growth affecting

the lymph glands or other lymphadenoid tissues." Unna (9) evidently considers a lymphoma to be simply a collection of leucocytes, for he says, "we shall not go far astray if we regard this very obstinate form of leukæmic cutaneous nodule also as a granuloma and not as a collection of leucocytes—a lymphoma."

With gratifying clearness Senn (10) defines lymphoma as "a benign tumor formed of lymphoid tissue produced from a matrix of lymphoblasts;" further (11), as "an encapsulated tumor which manifests no tendency to implicate adjacent glands and which is never complicated by affections of the blood-forming organs." That this conception of a lymphoma was largely theoretical in its deduction is evidenced by the admission of the author that "it is easier to say what a lymphoma is not, than what it is." The reason for this last statement obviously lies in the absence from medical literature of any observations of growths which would conform to such a definition, *i. e.* a true tumor, benign, atypical in structure, yet representing lymphoid tissue and not due to any inflammatory process. Not even Senn refers to any such observations; he remarks further, "In no department of surgical pathology do we meet with more confusion than in the difference between benign and malignant tumors and inflammatory swellings of the lymph glands." This confusion is due not so much to a lack of knowledge of the inflammatory affections of lymph glands nor to an ignorance of the nature and characteristics of malignant tumors but to the great rarity of benign new growths of lymph glands.

I am indebted to Dr. J. B. Murphy for the opportunity to examine a small tumor, which, I became convinced from a careful study, represented a benign, non-inflammatory, new-growth of lymph nodes; in other words, a lymphoma according to Senn, a benign lymphoma according to Ziegler and others.

The tumor was located in the left groin and had attained the size of a pigeon's egg by a very gradual growth extending over a period of fifteen years. The inguinal canal was not patent and there was no enlargement of the adjacent glands nor of those of the opposite side. The man was in good general health; there were no similar

tumors elsewhere on the body, and there has been no recurrence of any growth since its removal three years ago.

When examined in the fresh condition the tumor presented an oval, flattened shape not very unlike a small kidney (Plate XXVI, Fig. 1). On section, light reddish surfaces were exposed which were studded with areas the size of millet seeds and resembling closely the Malpighian bodies of the spleen. These minute areas were very uniform in their distribution; there was apparently no difference in this respect between the cortical and the more central portions, these two regions being quite similar in color. Although a fibrous capsule surrounded the growth, there were no coarse trabeculæ entering the tissue. The blood-vessels exposed in a number of sections were all small. There were no spots of softening or hæmorrhage nor any special features which would point to an inflammatory process.

Microscopic Examination.—The tumor was hardened in alcohol. Sections stained by ordinary methods show the tissue to be distinctly lymphoid in character and to contain many lymph nodes (Plate XXVI, Fig. 2). In serial sections the largest diameters of one of average size were 112 by 88μ. A very large node measured 160μ in its longer diameter by 116μ in the shorter. The cells composing these nodes are arranged in a very striking and peculiar manner. Around the periphery they form encircling rows which extend for one-third or, in rare instances, one-half of the circumference (Plate XXVII, Fig. 4). The arrangement in rows is more perfect in some nodes than in others, but when serial sections are examined it is found to occur at some point and to some degree in every node. Frequently these rows are double; or three or four rows form a band, which, extending around the node for some distance, breaks up to anastomose with similar bands or single rows. Between these rows and bands are cleft-like spaces (Plate XXVII, Fig. 4) which are more marked in some preparations than in others, and in some are undoubtedly to be ascribed to shrinkage. The rows are most perfect at the periphery, so that the edge of the node is usually well defined. This is, however, not invariably the case, for some nodes fuse gradually with the internodal tissue, while short tangential rows shoot out into the surrounding

tissue, obscuring the distinctness of the margin. The cells themselves which form these rows are small, 4 to 5 μ in diameter, with a nucleus which occupies almost the entire cell, leaving only a narrow margin of cytoplasm (Plate XXVII, Fig. 6). The nuclei are round, with a deeply staining nuclear membrane. The chromatin frequently forms an irregular lining for the nuclear membrane and usually two or three centrally located large granules.

The reticulum supporting these cells is very scanty in the outer zones of the node. Here it forms delicate strands which extend between and parallel to the rows. Toward the centre it is more dense and forms heavier bands. It is always greater in amount and, in carefully pencilled preparations, is found to produce a finer meshed network around the blood-vessels.

Blood-vessels can be found in every node by the study of serial sections, and there are generally one or two in the majority of the sections from any one node. The vessel enters obliquely and frequently divides after its entrance. In such cases its further course is not easily followed. These vessels are evidently arterioles; they possess thick hyaline walls. In case the blood-vessel is cut directly across, the row formation is very perfect; row after row or layer upon layer surround the vessel throughout the entire nodule. When the vessel is cut obliquely the row arrangement is more regular at the periphery, the remainder of the node consisting of the same small darkly staining cells grouped in a dense mass and devoid of any formation of rows. Figure 3 of Plate XXVI shows the rows of lymphoblasts about one of the larger internodal blood-vessels.

Each node is found to possess at some point a group of cells which afford a marked contrast to those just considered. This group is generally central in its location but in some sections is nearer to one pole of the node (Plate XXVI, Fig. 2, and Plate XXVII, Fig. 4). The cells forming these groups are large, 8 to 10 μ in their long diameter, and like the others consist mainly of nuclei. But these nuclei are oblong and irregular or notched and stain faintly with the exception of the nuclear membrane. In examining successive sections one reaches the conclusion that these groups of pale cells occur at or near

the point where the arteriole breaks up to form capillaries, and, further, that the individual cells resemble in size, shape and staining those which line the blood-vessels. The division into capillaries results in a denser reticulum which, with the large faintly staining cells, forms an area differing very much from the densely packed, darkly stained cells which surround it. When, as exceptionally is the case, a rather large arteriole passes into a node and immediately divides into a number of small branches, the resulting pale centre is quite large.

Thus it is seen that the light areas resembling the Malpighian corpuscles of the spleen, observed when the tumor was first cut, are lymph nodes which are remarkable for their extraordinary size and the extreme regularity in the arrangement of the cells forming them; also that these nodes are formed around blood-vessels or at points where the blood-vessels divide to form capillaries.

The internodal tissue is loose and shows considerable stroma, which supports small cells similar to the darkly staining cells of the nodes. There is no particular arrangement of these except that they are more numerous at the edges of the spaces formed by the reticulum, the centres of such spaces being empty. The reticulum of this internodal tissue, stained by Van Gieson's stain (Plate XXVII, Fig. 5), picronigrosin or the iron-hæmatoxylin method and in carefully pencilled sections, shows no nuclei which can be positively said to be a part of the stroma in spite of the fact that the nuclei of the cells lining the spaces often appear as though they belong to the reticulum, as though in fact the reticulum were connected with cells. The blood-vessels in the internodal tissue are larger than any found within the nodes. The arteries possess thick hyaline walls with two, three or more elastic sheaths and occasional isolated elastic fibres (Plate XXVII, Fig. 7). The thickening in the walls of the arteries and arterioles has affected only the tunica media. As the vessel becomes smaller its wall becomes more and more homogeneous and hyaline in character, until finally no nuclei can be seen in its middle coat. The veins are easily recognized by their thin walls; they show no changes. Blood is quite generally absent from the vessels, a small amount occurring in the

largest. There are no heavy bands of stroma which extend in from the capsule. The capsule itself is thin, largely fibrous, with few nuclei.

The similarity of the nodes, which form the greater and more interesting part of this tumor, to the " secondary nodules " of lymphoid tissue described by His, Flemming and others is very apparent.

Hoyer (12), in describing the cells in lymph glands, referring to the pale centres of the germinal areas, speaks of " the cells described by Flemming with large nuclei which give the centres of the germinal areas their pale color."

Hoehl (13), in a good description of the reticulum of lymph glands, states that the connective tissue surrounding the germinal areas is arranged in a tangential direction as regards the periphery of the nodes, and Flemming (14) says that in the germinal areas it must be that there is a form of slow centrifugal pressure whereby the younger cells are pressed toward the periphery and driven out through the spaces of the reticulum. It can be seen from these citations and from the structure of the tumor that cells were produced in these pale centres and pressed toward the periphery, but lacking a normal exit through lymph sinuses into the circulating blood were accumulated in the growth and produced not only a remarkable formation of rows around such germinal centres, but also extremely large nodes. Concerning the formation of lymph nodes about blood-vessels, Flemming (15) finds that " the view that the arrangement of the lymph node about the blood-vessel originates in the germinal area, is perfectly acceptable." Also Ribbert (16) has noted that lymphomata occur on blood-vessels without any increase in the number of leucocytes in the blood.

Hansemann (17), in a consideration of the tumors which may develop from lymph glands, specifies three varieties of cells from which they may originate, viz.: the lymphocytes which form the parenchyma proper, the endothelial cells and the cells which form the framework (Gitterzellen). The existence of the last is positively denied by Hoehl (18), who says " the reticulum is entirely devoid of nuclei."

Senn (19) tells us that in true lymphoma " the follicular structure

(nodes) is well preserved," and that "the lymphoblasts produce lymph corpuscles which are not transformed into leucocytes but remain in the reticulum of the tumor as the essential cell elements."

It is well to note the resmblance in structure of the nodes in this tumor to the corpuscles of the spleen, for Albrecht (20) has reported a case in which numerous dislocated spleens were scattered over the peritoneal surfaces. Their number was estimated at about four hundred, and they varied in size from those which were strictly microscopic to some the size of a walnut. The greater number were found in the upper left quadrant of the abdominal cavity and on the posterior surface of the greater omentum. But they were found also in Douglas's pouch and on the upper one-third of the rectum. Eight were examined microscopically and found to resemble splenic tissue in every respect. They contained large quantities of blood and blood pigment, and in one of the larger examples typical splenic follicles were found about the blood-vessels.

The absence of blood pigment, as well as the scanty amount of blood and the lack of coarse trabeculæ would, in themselves, be sufficient to prevent a conclusion that the above-described growth represents or has sprung from displaced splenic tissue. The pale germinal areas present in the nodes of the lymphoma and absent from splenic follicles afford another striking difference. Albrecht also found accumulations of round cells about the masses of blood pigment, whereas the absence of any of the histological features of inflammation in this growth constitutes one of the main reasons for concluding that it is a true lymphoma.

Freund (21) has called attention to the resemblance between the accumulations of cells around the blood-vessels in periarteritis nodosa and lymphomata as follows: "Indeed one sees not seldom circumscribed areas of cells in the adventitia which have some similarity to lymphomata." In periarteritis nodosa, however, many vessels of the body and in different sites are the seat of such accumulations. The clinical picture is often that of a toxic neuritis or a toxic myositis and not only is the process in and around the vessel wall histologically an inflammatory one, but there are also changes in the organs due to obstruction of the vessels.

In concluding, this tumor seems to form a veritable confirmation of the words of Lancereaux (22), who in 1875 wrote: "It is indeed probable that this lymphoid tissue gives birth to young cells, and one can see how, under the influence of a mild irritant, the production of these cells becomes more abundant; the elements of the network multiplying at the same time, it results that tumors occur which have for their common characteristics a reticulated network in which the lymphoid elements are accumulated more or less abundantly. Such tumors or lymphatic growths are designated by the name—lymphoma."

DESCRIPTION OF PLATES XXVI AND XXVII.

PLATE XXVI.

Fig. 1. Photograph of lymphoma (exact size). The tumor has been cut open and from the right half portions have been removed for examination.

Fig. 2. Photograph of section showing lymph-nodes and internodal tissue. Low power.

Fig. 3. Formation of lymphoblasts about one of the larger internodal blood-vessels.

PLATE XXVII.

Fig. 4. Photograph of centre of a node showing the pale germinal area.

Fig. 5. Reticulum of the internodal tissue. Pencilled section; Van Gieson's stain.

Fig. 6. Cells of the rows of lymphoid tissue.

Fig. 7. Cross-section of one of the larger internodal vessels showing the elastic sheaths. Orcein stain.

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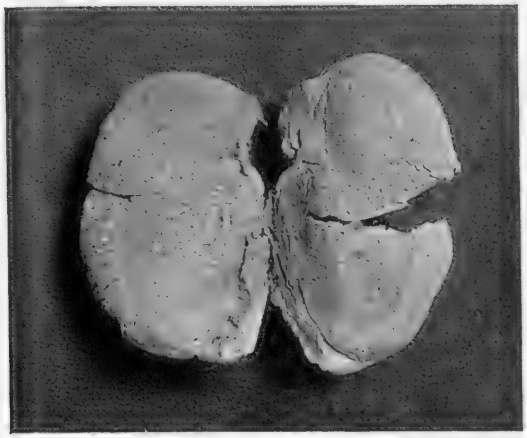

FIG. 1.

FIG. 3.

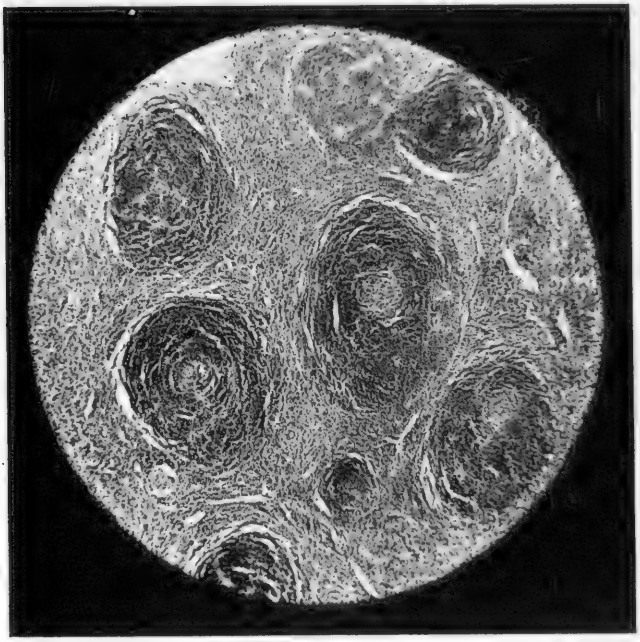

FIG. 2.

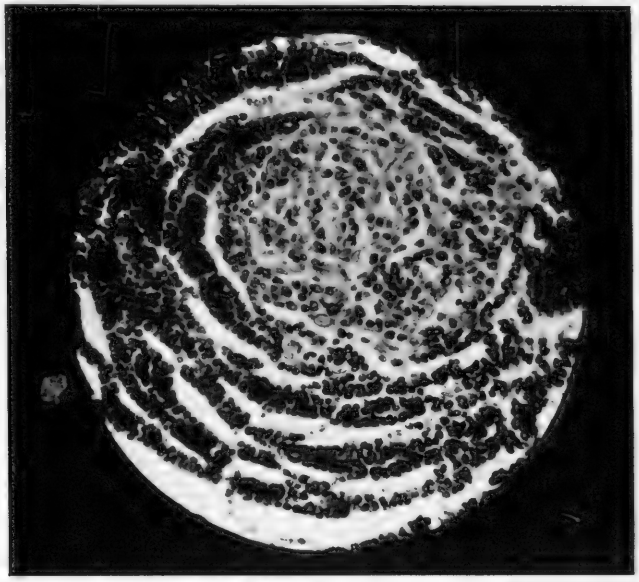

FIG. 4.

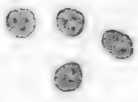

FIG. 6.

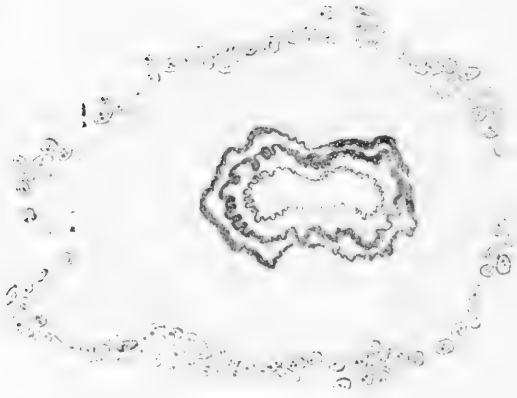

FIG. 5.

FIG. 7.

OBSERVATIONS CONCERNING LEUKÆMIC LESIONS OF THE SKIN.

By HORST OERTEL.

(From the Pathological Laboratory of the University and Bellevue Hospital Medical College.)

Some time ago I published in the *Yale Medical Journal** a preliminary report of a case of leukæmia cutis. I present now a more extensive study of this case. For the sake of convenience much of the matter of the previous article will be included here.

The rarity of leukæmic cutaneous affections may be inferred from the fact that a number of text-books on medicine and even on dermatology either do not speak of this condition at all, or simply mention it in a general way. In Unna's book† on the pathology of skin diseases there may be found a critical summary of 9 cases, and while perhaps a few may have escaped detection, probably scarcely more than a dozen cases are on record. Kaposi's case (1885) is the most frequently cited, and has directed especial attention to these lesions; the first observation, however, goes back to Biesiadecki's report in 1876, whose article is often quoted also with reference to the theory of leukæmia. The leukæmic affections of the skin which have hitherto been observed may be grouped into three classes.‡

The first class is represented by cases in which there are circumscribed, multiple, pinhead to hazelnut-sized, rapidly growing, pale or faintly red to brownish-colored tumors, irregularly distributed over the body, with little tendency to retrograde metamorphosis or ulceration. To this group belong the cases of Biesiadecki, Hochsinger and Schiff, Oliver, and Philippert. Hochsinger and Schiff found in their

* January, 1899.

† Unna, Die Histopathologie der Hautkrankheiten. Berlin, 1894.

‡ In this classification I depend upon Unna's presentation of the subject. I therefore omit the bibliography contained in his book, *op. cit.*, p. 619.

case cellular infiltrations, situated especially between cutis and hypoderm, which had changed the superficial fat layers to lymphoid tissue, and closely surrounded the glands. The infiltration started from the capillaries of the coiled tubes and the superficial fat layers, and consisted of lymphoid cells without spindle or giant cells. The upper part of the cutis and the papillary portion were almost free from infiltration except where, by constriction from above, the cutis would meet the infiltration. The nodule did not contain any blood-vessels; at the periphery these were dilated, being distended with blood. The coiled tubes were well preserved within the nodule. In Oliver's case there were 60 subcutaneous nodules presenting the structure of a round-celled sarcoma. Philippert observed subcutaneous, brownish-colored, smooth tumors on the face and head, appearing later in the nasal and pharyngeal mucous membranes. They possessed an adenoid structure. In a case of acute leukæmia described by Seelig,* the skin behind the ears and in different situations over the thorax and abdomen showed, three days before death, small, freely movable nodules, and small hæmorrhages. Microscopically the nodules were composed of lymphoid tissue, embedded in the subcutaneous fat, and containing small lymphocytes and larger uninuclear cells, the whole surrounded by a delicate connective tissue capsule.

The second class is characterized by a few, solitary, brown, markedly elevated, lobulated, firm, slowly growing, and persistent nodules. It is illustrated by Neuberger's case, according to whom the condition consists of a small lymphoid-celled infiltration into the cutis, especially around the follicles, by which the deeper layer of the epidermis is compressed, although it is still separated from the infiltration in the lower part of the cutis by a small, narrow, non-infiltrated zone of connective tissue. The surroundings of the coiled tubes, the hypoderm, and the muscular tissue of the cheeks were also infiltrated. Neuberger regards the coiled tubes, which themselves remain intact, as the starting point. Around them the lymphomatous formation appeared more distinct than in the upper parts of the cutis.

The third class, finally, is represented by cases of which that of

* *Deutsches Arch. f. klin. Med.*, 1895, liv, 537.

Kaposi may serve as a type. It is a diffuse form, showing on the one hand a diffuse, moist, eczematous appearance, especially of the head, extremities and chest, and on the other hand tumors the size of a pea to a pigeon's egg, which ulcerate spontaneously, leaving a large, flat, red ulcer. An œdematous condition of the skin is present. Sections in Kaposi's case showed a marked œdema of the hypoderm, which could be distinctly differentiated from the healthy yellow adipose tissue. The interstitial tissue between the fat lobules had sclerosed and traversed the œdematous masses in the form of thick white septa. The œdematous could not be distinctly separated from the healthy parts surrounding them. The cutis above the nodule was œdematous, or reddened and ulcerated, or confluent with the nodule and of an amyloid appearance. Microscopically these tumors consisted of a non-vascular, fine stroma, with dense leucocytic infiltrations, most prominent in the region of the coiled tubes. The hair follicles were unchanged. The rete Malpighii showed partly a proliferation, partly a rarefaction, produced by the approaching infiltration. Galliard's earlier observed case is very similar. He found a reticulated formation, pronounced around the sweat and sebaceous glands, and between the fat lobules. In the more recent nodules the superficial layers were relatively undisturbed. In Leber's, and Chavel's cases the eyelids especially were the seat of a diffuse infiltration. Here also the microscopic examination revealed small cells in a fine reticulated stroma.

In a patient suffering from lympho-lienal leukæmia, exhibited by Litten* to the Berliner medicinische Gesellschaft, the upper eyelids presented a swollen appearance suggestive of œdema, but which closer examination showed to be due to small lymphatic tumors, over which the skin was freely movable. No microscopical examination was reported. In the discussion Köbner commented upon the extreme rarity of leukæmic affections of the skin and remarked that lymphomata, which may cause a similar condition, extend, in contrast with leukæmic tumors, into the subconjunctival tissue.

Kelsch and Vaillard's† case of multiple, metastasizing, malignant

* *Deutsche med. Wochenschr.*, 1897, Vereins-Beil., i, p. 3.
† *Ann. d. l'Inst. Pasteur*, 1890, iv, 276.

lymphomata, appearing in the skin near the eyes, and also in the thyroid, abdomen and bones, although interpreted by them as leukæmic, was not, as pointed out by Schmorl,* demonstrated to be an instance of leukæmia, and probably does not belong to the class of affections now under consideration.

From the foregoing it appears that the leukæmic dermatoses have in common a small-cellular circumscribed or diffuse infiltration, which originates in the deeper layers of the skin and extends upward.

Little attention, however, has been given to the more detailed study of the characters of the cells making up this infiltration. While the formation is regarded by most observers as a lymphoma, this idea is apparently based more upon the general characters of the case than upon any definite investigation of the tumor. The term "small-celled infiltration" is an exceedingly indefinite and wide one, applying to pathologically entirely different conditions, so that, whenever it is possible, more definite and precise descriptions should be given. It is for this reason that Unna doubts that these structures really represent lymphomata. He is inclined to regard them as granulomata, containing plasma cells, especially as he believes that they present in their circumscribed foci the characteristics of such growths.

The nature of leukæmic affections of the skin has been recently discussed with much fulness by Nékám,† who reports a typical case, but before considering his views it will be well to state the opinions held concerning the characters of the secondary leukæmic nodules which are observed with much greater frequency in other organs of the body, especially the liver, than in the skin.

Virchow‡ regards the leukæmic nodules, which may appear in the liver, kidneys and other situations normally devoid of lymphatic tissue, as true neoplasms. He points out that the new formation may appear in the form either of an infiltration or of definite nodules and says: "A kind of new lymph-glands may thus be developed within an organ which otherwise contains nothing of the sort. . . . We possess

* *Centralb. f. allg. Path. u. path. Anat.*, 1891, ii, 118.

† Die leukäm. Erkrankungen der Haut. *Monatschr. f. prakt. Dermatologie*, Ergänzungsheft, 1899, ii.

‡ Die krankhaften Geschwülste, ii, p. 568 et seq. Berlin, 1865.

in leukæmia a very complete picture of the gradual generalization of an originally local process, in which we can trace the individual steps more definitely than in any other kind of generalization."

A similar interpretation of leukæmic nodules as the seat of active formation of new cells is advanced by Bizzozero,* who differs from Virchow, however, in regarding emigration from the blood as the primary source of the leucocytes in these secondary nodules. But Bizzozero does not find that the nodules are merely passive accumulations of migrated cells, for he was able to demonstrate not only in the hyperplastic spleen, lymphatic glands and intestinal follicles, but also in the secondary nodules in the liver and kidney, abundant karyokinetic figures within the lymphoid cells. This abundance of nuclear figures is, according to Bizzozero, one of the marks distinguishing these leukæmic nodules from the accumulation of leucocytes in suppurative foci.

Similar observations as to the occurrence of cell proliferation in leukæmic nodules in internal organs have been made by M. B. Schmidt† and by Hindenburg,‡ both of whom note the presence of indirect cell division also in the capillary endothelium, particularly in the liver. These writers, therefore, agree with Virchow's conception as modified by Bizzozero.

In contrast to the preceding views is that held by Rindfleisch,§ Cornil and Ranvier|| and Löwit,¶ who regard the secondary leukæmic nodules and infiltrations as merely passive accumulations of leucocytes derived by migration from the blood. Ziegler** attributes the nodules to migration of cells from the blood followed by local proliferation of cells in the foci. Klebs†† places leukæmic nodules under the head of his leukocytomata and allies them with tubercle, leproma and other infectious granulomata.

* Virchow's *Archiv*, 1885, xcix, 378.
† Ziegler's *Beiträge*, 1892, xi, p. 228 et seq.
‡ *Deutsches Arch. f. klin. Med.*, 1895, liv, 209.
§ Lehrb. d. path. Gewebelehre, Leipzig, 1886, p. 497.
|| Manuel d'histologie pathologique, i, p. 296. Paris, 1881.
¶ *Situngsb. d. k. Akad. d. Wissensch.* (Wien), 1886, xcii.
** Lehrb. d. spec. path. Anat., p. 9, Jena, 1898.
†† Allgem. Pathologie, ii, p. 597, Jena, 1889.

The most precise statements hitherto recorded concerning the composition of leukæmic cutaneous nodules are to be found in Nékám's* recent discussion of the subject. A critical review of the records has convinced him that a number of cases reported as examples of leukæmia cutis, and especially the diffuse lesions falling under the third group (lymphodermia perniciosa), cannot properly be regarded as such inasmuch as neither the clinical nor the anatomical characters of leukæmia or of the tumors were indisputably established. He accepts as undoubted examples the cases of only Biesiadecki, Hochsinger and Schiff, Neuberger, and himself, in all of which there were definite nodules the size of a pea to a hazelnut without a diffuse appearance. Nékám thus describes the process: "The condition starts with an œdema of the skin, followed by a diapedesis of all the constituents of the blood along the course of the larger blood-vessels. This infiltration appears rapidly in the tissues surrounding the vessels of the coiled tubes, the superficial fat layers, the cutaneous muscles, and the follicles, and extends in an anatomical direction, but in a somewhat irregular manner, diffusely, or in streaks, or in lymphoid masses. The cells are chiefly lymphocytes, very infrequently plasma cells. Mitoses, giant cells, newly-formed blood-vessels, and a change of lymphocytes into plasma cells could not be seen. The cells in the centres of the nodules persist unchanged. Now, inasmuch as in all probability the process of emigration of cells from the blood is a continuous one, whereas the growth of the nodules is very slow, attaining, even in years, only small dimensions, it follows that cells must disappear from the nodules. In support of this view is the fact that red corpuscles and eosinophilic cells are found only in recent nodules. . . . The red corpuscles are removed by the lymphatic vessels. Other cells are destroyed in the subpapillary layers and their granular fragments appear in the rete Malpighii and on the surface of the skin. This may be regarded as a useful elimination of leucocytes. . . . These observations make it evident that leukæmic tumors of the skin must be regarded as infiltrations, and we can, therefore, accept as such only those which fulfil the following conditions: (1) They must

* *Loc. cit.*

occur during the course of true leukæmia; (2) their origin must be exclusively a diapedesis of cells from the blood, local proliferation of cells being absent; (3) a part of the cells, especially the red corpuscles, are returned to the blood by way of the lymph-channels, another part, especially the leucocytes, are carried off through the epidermis, or otherwise discharged; (4) the chief bulk of the cells composing the nodules undergo no metamorphosis. The atrophy of the normal connective tissue leads to the formation of a rarefied network in which the lymphoid cells lie embedded, and this adenoid character of the tissue persists for years. The infiltration starts around the deeper vessels and extends rapidly upwards into the papillary layer." *

For the study of the case which I here report I was not able to make any prolonged observations during the life of the patient and I encountered difficulties in obtaining sufficient material for as thorough histological investigation as I desired.

History.—The patient was a man of about 40 years, unmarried, who had been in fairly good health until about two years before, when he was first taken ill with "various digestive disturbances and malaria." The attending physician, who treated him from that time on until his death, states that at that time he had an irregular intermittent fever and an enlarged spleen, which he regarded as an amyloid (?) spleen consequent to malaria. This "malaria," which finally subsided, was characterized by a stubborn resistance to quinine, and arsenic was, therefore, given in addition. The patient, however, recovered sufficiently to attend to his duties as a clerk. His appetite was of the best, and indeed continued so until two days before death, when violent vomiting set in. Several months (how many cannot now be determined) before this occurred, his trouble became aggravated; the spleen enlarged still more, the patient emaciated, had irregular fever, and "a long, hard, inflammatory tumor appeared in the region of the right rectus abdominis." This was diagnosed as a myositis and treated with ichthyol, under which treatment it disappeared. Up to this time the diagnosis had been malaria; no blood examination, however, had been made. A few weeks later the attending

* It may here be stated that various affections described, particularly by French writers, under the designation of cutaneous lymphadenia or lymphadenoma, and including manifestly diverse affections, are not considered in this article and, at least for the most part, have nothing to do with true leukæmia.

physician noticed that "there appeared on the skin small nodules, irregularly distributed over the body." They seem to have been observed first on the chest and arms, then on other parts of the body. (See protocol of autopsy below.) Details about their clinical appearance, and how rapidly they grew I was unable to obtain. Their appearance at once led to a diagnosis of multiple sarcoma of the skin. It was subsequently decided to send the patient to New York to a noted specialist for treatment with streptococcus serum, but he was sent back as a case no longer suitable for this treatment. The patient was then referred to me. I suggested a blood examination as imperative, and a blood slide was brought to the laboratory. The picture which it showed agreed well with the clinical history. The enormous increase and the special characters of the white blood corpuscles were at once evident. This unstained specimen showed, besides polynuclear cells, many large mononuclear cells of the type of myelocytes, and a marked increase of eosinophilic cells. A second specimen for staining was requested, but was never obtained, and no opportunity was afforded for making an accurate blood count.

Nothing more of the case was heard until a few weeks later, when I was asked to make the post-mortem examination. Unfortunately the report of this must be nearly as meagre as the preceding history, as the autopsy had to be made in the parlor of the house, without any accommodations, and the bad local custom of injecting a foul-smelling liquid into the abdomen had been promptly followed. Since I was allowed to open only the abdominal cavity, the heart and lungs had to be extirpated from below. The removal of any organ was at first prohibited, but finally I obtained permission to take the spleen, a small piece of the liver, and two of the cutaneous nodules from the left thigh.

Protocol of autopsy (abstracted).—Body of a man apparently between 35 and 40 years old; about 160 cm. tall; extremely emaciated, no panniculus adiposus; muscles atrophied. No rigor mortis. Skin pale. Face shows extreme emaciation; cheek bones very prominent, lips pale and delicate. Scattered irregularly over the body as far up as the neck, numerous on arms and chest, but most marked on the thighs, are small, round, pale, hard nodules, varying from one to two centimetres in diameter, some elevated, all freely movable over the subcutaneous tissue. On incision they show a yellowish-white surface. Chest small, abdomen distended.

On opening the abdominal cavity a large amount of embalming fluid escapes. Small and large intestines distended; serosa pale, opaque, with injected vessels in a few places. The large omentum is very thin and

has everywhere formed thick, firm, fibrous adhesions* with the intestine and rest of the peritoneum.

Quite evident at once is the enormous spleen, which fills the entire left side of the abdomen, extending 8 to 10 cm. below the umbilicus and to the middle line. It is bound by very strong adhesions to the liver and diaphragm, pushing the heart upwards. On being removed, it proves to be 30 cm. long, 17 cm. broad, 6 cm. thick; its weight is 3.0 kg. It is of a dark bluish color and dense consistence. The capsule is very much thickened. On incision it shows a dark red surface; in several places are yellowish spots, the size of a pin's head; at the periphery are a few yellowish, wedge-shaped areas, the bases of which are about one centimetre broad. With the tissues around the spleen an accessory spleen of the shape, size, and color of a plum is removed.

The left kidney, with a fairly extensive fat capsule, is larger than normal and firm; capsule adherent; surface smooth, white, with bluish-red and yellow spots. Cortex broad, pale, dotted with spots of the same character. Pyramids reddened. Right kidney: same condition.

Liver larger than normal; firm; of pale yellow-brownish surface; on section pale yellow; acini distinct.

Heart the size of the man's fist; right side moderately covered with fat; walls normal in thickness, with dark brownish areas; valves normal.

Lungs: in upper portion of a dark red color; the lower portion presents a grayish appearance; emphysematous; the tissue has a crepitant feel, but is soft; on section a large amount of a clear, frothy liquid escapes.

Anatomical diagnosis: Leukæmia; great enlargement of spleen; old splenic infarcts; chronic parenchymatous and hæmorrhagic nephritis; fatty liver; chronic adhesive peritonitis; pulmonary emphysema and œdema; brown atrophy of the heart; circumscribed cutaneous nodules.

The two cutaneous nodules removed at autopsy were fixed in ten per cent formalin solution, which proved very successful, hardened in alcohol, and embedded in paraffin. For a preliminary study specimens were stained in the common way with hæmatoxylin and eosin, later after Biondi-Heidenhain and with methylene-blue according to Unna.

The epidermis and the upper part of the cutis showed no definite changes. The lower part of the cutis, however, was the seat of a

* The "hard long inflammatory tumor in the region of the rectus abdominis," diagnosed as myositis, was undoubtedly one of these strings of thickened peritoneum. I was told that shortly before death several of these masses could be felt and were then regarded as sarcomatous metastases.

dense and diffuse so-called small-celled infiltration. This infiltration extended upward, penetrating the fibres of the cutis, either replacing them entirely or separating them into bundles. Only a very fine reticulum of connective-tissue fibrils remained, which contained greater or smaller groups of cells. In this way some parts of the superficial fat layers were entirely replaced by this infiltration, while others were left relatively undisturbed. Connective-tissue sclerosis, newly-formed blood-vessels, giant cells and necrotic areas or other regressive metamorphoses of cells were absent. The glands were embedded in the cellular masses; they did not show a direct relation to the process. In the further examination of a greater number of sections, especially of one of the nodules, which apparently was more recent, several stages of the process could be demonstrated.

The earliest changes were represented by small, circumscribed focal accumulations of cells situated in the lower part of the cutis. These foci were distinct even to the naked eye, in the microscopic sections, as whitish, tubercle-like formations. As the process progressed, these small nodules became confluent and the appearance then was that of a diffuse infiltration. This was particularly well seen in specimens stained after Biondi-Heidenhain.

In the hæmatoxylin-eosin preparations the largest number of cells possessed irregular nuclei, lobulated and very rich in chromatin, having all the appearances of leucocytes. Only a small number were characterized by fainter, vesicular nuclei, with distinct nucleoli. These latter were regarded as connective-tissue nuclei.

A more careful study of the cells was made in the sections stained after Biondi-Heidenhain and with Unna's polychrome methylene-blue. The largest part of the infiltration was made up of cells presenting the characters of lymphocytes. Of these two kinds could be distinguished: (1) small cells with narrow, faintly staining cytoplasm and deeply staining nucleus, and (2) larger cells with feebly staining cytoplasm and a large, sometimes indented, somewhat irregularly staining nucleus. Besides these two varieties of cells (3) polynuclear leucocytes were common as well as (4) cells with bright red coarse granulations (α-granulation, eosinophilic cells). The large number of

eosinophilic cells deserves especial attention in the light of Nékám's statement that they are found only in recent nodules. In this patient the cutaneous lesion did not appear until a few months before death, and the two nodules taken from the thigh for examination were apparently of recent formation. If red blood corpuscles escape in any large amount, they must certainly be removed very rapidly, for they were not at all conspicuous in my specimens. In only a few sections did I occasionally see a large cell with large eccentric nucleus which answered to the characters of plasma cells. But these cells were compressed and their view obstructed by the great number of surrounding leucocytes. Plasma cells, if present at all, were certainly in such small number as to form no characteristic part of the cellular accumulations. Mitoses were not observed, and while it is desirable to examine material from a larger number of cases before passing a final judgment upon their occurrence in leukæmic cutaneous nodules, their absence in both Nékám's and my cases indicates that they are not constant or essential features of these nodules.

In view of Unna's statements concerning the nature of leukæmic nodules in the skin, the absence of plasma cells, at least in noticeable quantity, is of interest. It may be recalled that Unna, who first distinguished these cells, believes that they are derived from connective-tissue cells, but the subsequent studies of Marschalkó, Justi, and Councilman* have demonstrated that they come from lymphocytes. Plasma cells are found especially in infectious granulomata, in granulation tissue, and in many of the so-called inflammatory round-celled and lymphoid-celled infiltrations of organs. We possess no information at present concerning the occurrence of plasma cells in leukæmic nodules in the liver and other parenchymatous organs. Their absence from these nodules in the skin, noted in Nékám's and my cases, is noteworthy, in view of the predominance of lymphocytes in these lesions, and, taken in connection with the absence of mitoses, speaks against the occurrence of active cellular growth and multiplication in any considerable degree within these nodules. As already

* *Journal of Experimental Medicine*, 1898, iii, 393. Consult also for other references and a full consideration of these cells.

indicated, the connective tissue is the seat of atrophy at the site of the cellular accumulations, and does not participate by proliferation of the fixed cells in the formation of the nodules. It furnishes a kind of reticulated stroma for the cells.

The histological examination of the leukæmic cutaneous nodules in my case, therefore, supports the view of those who regard secondary leukæmic nodules as essentially composed of cells derived from the blood. I am not inclined to lay great stress upon the failure to find karyokinetic figures in the cells, as indicative of a distinction between these nodules in the skin and those so often found in leukæmia within parenchymatous organs, but upon this point further observations are needed. Why the skin should be such an uncommon seat of secondary leukæmic nodules, thus contrasting with the liver and kidneys, it would be at present useless to speculate.

The propriety of designating these nodules as lymphomata cannot be profitably discussed until there is greater agreement of opinion, than at present, concerning the definition of what shall be called "lymphoma." As this term etymologically suggests an actual new growth or tumor, its application to such nodules as those described in this paper seems to me of at least questionable propriety, as it is clear that these nodules are not genuine neoplasms.

I desire to express sincere thanks to Professor Dunham of the University and Bellevue Hospital Medical College for kind assistance in the preparation of this article and to Professor Welch of the Johns Hopkins University for helpful suggestions.

A CASE OF ADDISON'S DISEASE WITH SIMPLE ATROPHY OF THE ADRENALS.*

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(*From the Pathological Institute of the New York State Hospitals and the Carnegie Laboratory, New York.*)

Plate XXVIII.

The study of simple atrophy of the adrenal bodies in Addison's disease may prove of considerable importance in explaining more thoroughly the process underlying this group of symptoms. The lesions in simple atrophy of the adrenals afford a much better opportunity of investigating the gradual death of the parenchyma than the gross destructive changes in tuberculosis of the glands, most commonly associated with the disease. The histological examination of the comparatively early phases of the atrophic lesions in the present case possesses considerable interest, for the subject has received but little investigation. Very few cases of simple atrophy of the adrenals in Addison's disease are recorded, and of these hardly a single one has been given thorough histological study.

I am indebted for the following clinical notes to Dr. D. H. McAlpin, Jr., Visiting Physician to the City Hospital of New York.

F. N., male; aged 42; admitted to City Hospital August 10, 1897, died February 17, 1898.

Family history: Negative.

Previous history: During childhood the patient had measles. At 20 years of age he had an attack of rheumatism, after which he was well until his 28th year, when he had some affection of the skin. Patient denied alcoholic excess and syphilis.

Fourteen years ago, he says, he began to "turn yellow," but he was well for seven years after the first appearance of the discoloration, when he

* Read before the New York Pathological Society, February 8, 1899.

had another attack of rheumatism in the legs, from which he soon recovered. In 1895 he had a similar attack of "rheumatic" pain in the legs, accompanied by acute bronchitis, loss of flesh, and profound weakness, but he recovered and was apparently well until June, 1897. Six weeks before admission, he had an attack of weakness, diarrhœa and polyuria, without headache or pain in the abdomen. The feet became swollen and he had pains in the legs and ankles.

Status on admission: The entire body is of a yellowish-brown color, deeper in tint where protected from the sun, and especially dark over areas pressed upon by the clothing, waistband, collar, suspenders, etc. Ocular conjunctivæ pearly white. Mucous membrane of tongue and mouth pale and free from pigment. The patient's mental condition is dull. He says he has a fair appetite, sleeps well, but has pains in the knees and ankles. The bowels move three to four times daily. He complains also of epigastric pain and slight cough.

Physical examination of lungs revealed high-pitched and rather prolonged respiration at inferior angle of left scapula. Blood examination, September 6, by Dr. Reilly; hæmoglobin, 20 per cent; reds, 1,490,850; whites, hypoleucocytosis. From the stained specimens, I made the following observations: Differential count of 500 leucocytes gave: adult cells, 55 per cent; young cells, 41 per cent; eosinophiles, 4 per cent; myelocytes absent. There are a few microcytes and poikilocytes but the blood does not indicate severe anæmia.

September 11. Patient complained of severe darting pains from the neck to the occipital region and of pain in the left hypochondrium. For the past 18 days he has had frequent stools. At this time examination showed impaired resonance over both infraclavicular regions with fine moist rales, heard especially posteriorly.

September 13. On ophthalmoscopic examination, retinæ and optic disks were extremely pale, but otherwise normal. The following week the patient seemed much improved. The cough became less frequent and severe, the stools less numerous and he appeared to be brighter mentally. On September 22, diarrhœa recurred with frequent bilious vomiting, watery stools, cough, pain in the abdomen and legs. This condition persisted except at intervals of a few days during which the patient would feel fairly comfortable. On October 26, ptosis of the right eyelid developed, but disappeared after a few days.

On physical examination, November 5, bronchial breathing, coarse mucous rales, friction, etc., were heard over the right infraclavicular region, but there was no evidence of cavity formation.

The patient remained in this condition until January 15, 1898, after which date he began to grow much weaker. The diarrhœa and vomiting continued with severe abdominal pain and at times delirium. He died February 17, 1898. The temperature was irregular and atypical and at no time above 101° F. The urine was examined weekly. The specific gravity varied from 1018 to 1022. Neither albumin nor sugar was found.

The *autopsy* was performed a few hours after death. I have prepared the following notes from Dr. McAlpin's protocol.

Body extremely emaciated; eyes and cheeks sunken; panniculus adiposus absent. Skin of entire body of a brownish mulatto color, pigmentation being especially pronounced over upper anterior part of thorax, scrotum, glans penis, dorsum of hands and feet, and either side of spinal column, especially in lumbar and sacral regions. No pigmentation of buccal mucous membrane or sides of the tongue. Conjunctivæ generally white, but tinged with yellow where exposed to the air. Peritoneum smooth and glistening. Stomach and large intestine distended with gas. Lower edge of right kidney 1½ inches below last rib; left kidney same, slightly movable. Vermiform appendix small, its lumen patent and filled with impacted hard fæcal matter. Bladder distended. Edge of liver above free border of ribs; gall-bladder distended.

Right lung: Oedematous; a few old adhesions over middle lobe; cut section of lower lobe yellowish pink, with small, elevated, pale, dry, granular areas exuding a creamy fluid on pressure; upper lobe grayish yellow, with moderate anthracosis, and a few apical caseous nodules from 1 to 6 cm. in diameter.

Left lung: Oedematous; old adhesions over upper lobe; tubercular nodules at apex and a few in lower lobe, surrounded by firm, pinkish granulations. Bronchial glands enlarged and the right calcified.

Heart: About 20 cc. clear serum in pericardial sac. Heart of average size; epicardial fat absent; right ventricle collapsed; right auricle distended with ante-mortem clot; cavity of left ventricle dilated; walls thin, pale brown; endocardium normal; aortic and mitral segments practically normal; no myocarditis; right ventricle normal; tricuspid and pulmonary valves normal, save some yellowish discoloration of segments; also some atheroma of coronary arteries.

Liver: Small, reddish-brown, firm; lobules distinct, with dark centres; vascular walls thickened. Gall-bladder distended with two ounces of dark greenish, thick bile, containing many small, pultaceous masses; gall-ducts patent; mucous membrane normal.

Stomach: Small, mucous membrane normal.

Pancreas: Pale, very firm, evidently the seat of interstitial hyperplasia.

Small intestine: Extremely pale, distended with gas; mucosa pale, covered with mucus; no increase of adenoid tissue.

Mesenteric glands small, firm.

Spleen: Somewhat enlarged, firm, reddish-brown.

Bladder distended with eight ounces of dark urine, mucosa normal.

Kidneys: Enlarged; finely granular surface, in places cystic; cortex thickened; pale yellowish, striæ obscure, small, grayish, tubercle-like bodies on surface.

Adrenals: The left measures 2 in. in length by $\frac{3}{4}$ in. in width and less than $\frac{1}{4}$ in. in thickness; the right has about the same dimensions; their general appearance normal, except somewhat atrophic; surface smooth and pale; consistence normal; no evidence of tubercular disease.

Cœliac sympathetics easily removed and apparently normal.

Brain: Small, fresh hæmorrhages on under surface of dura, especially on right side. Pia mater very œdematous, opaque and thickened; in region of both Sylvian fossæ, dusky pigmentation, seen also over pons. Brain small, symmetrical and very pale; sulci deep, convolutions thin.

Spinal cord: Normal.

Anatomical diagnosis: Chronic miliary tuberculosis of lungs; tuberculosis of bronchial glands; passive congestion of liver; chronic interstitial pancreatitis; chronic catarrhal enteritis; chronic interstitial nephritis, and simple atrophy of adrenal bodies.

Microscopic examination: Unfortunately as the central nervous system was not preserved and the dissection of the sympathetic was incomplete, the microscopic examination is restricted to the only available material, the adrenal bodies and the semilunar ganglia.

Pieces from both *adrenals* were hardened in formaldehyde solution (4 per cent), in absolute alcohol, and in Müller's fluid. The entire gland of either side was cut and stained. The sections were variously stained in Delafield's hæmatoxylin and eosin-blue, lithium carmine, Van Gieson's mixture of picro-acid-fuchsin, by the Heidenhain-Biondi method, etc. The microscopical findings being similar in both adrenals, one description will suffice. On casual observation the appearance of many sections is that of normal tissue, but on closer examination and by careful comparison with a series of sections taken from normal adrenals distinct morbid changes can be demonstrated. The periglandular fat is decreased in amount and the seat of serous atrophy. The remains of the adipose tissue are seen as a richly cellular mass, in which appear fully formed fat

cells. The intercellular substance is a homogeneous finely granular material, in places fibrillated, which encloses small, round, deeply stained nuclei. The fibrous capsule, with the septa, does not appear thickened and is free from evidences of inflammation and of tuberculosis.

The zona glomerulosa is present in some part throughout all the sections, the individual glomeruli varying greatly in size and appearance. In places they are represented by only one or two small cells, with round, deeply stained nuclei, and at times they appear to lie in the outer capsule. In places, however, this zone appears two or three times the usual thickness and is composed of four or five layers of glomerular acini, and again in other areas it is entirely absent, the cells of the zona fasciculata being in contact with the fibrous capsule.

The part of the gland represented by the zona fasciculata shows many interesting as well as uncommon conditions. The most striking change consist in the irregularity of the columns in this portion. The cells, instead of being arranged in definite columns, running more or less vertically from the zona glomerulosa to the medulla, compose branching masses and columns, having an irregular course and being intimately mingled with cells from the zona reticularis. The cells vary greatly in size and appearance, their nuclei being small, round, deeply stained dots. The cytoplasm is finely granular and stains a delicate pink with a watery solution of eosin-blue. The walls of the individual cells cannot be distinguished, so that the general appearance is that of finely granular, branching masses, in which lie small, round nuclei. The small cells, which are arranged in tubules in this region, have protoplasm resembling in every way that of their enlarged associates. The fatty condition of the cells, normally present in this region, is absent or noticeably diminished and in only a few sections was I able to demonstrate the large, clear fatty cells, so common in the normal organ.

Especially in specimens hardened in Müller's fluid, are seen in the fascicular zone circumscribed areas composed of large, pale acini, containing a peculiar colloid material of crescentic and other shapes. These colloid masses, when stained by eosin, contrast by their brilliant red color with the pale pink cytoplasm. With picro-acid-fuchsin they are colored a brownish-purple. They have sharp borders and are striking objects in the sections. Auld describes cylindrical bodies with highly refractive index between, or more often in, the medullary cells of adrenals hardened with chromic salts. Colloid masses similar to those described, however, I have never seen in any other sections from a series of at least fifty adrenals studied during the past year.

Adjacent to the swollen areas of the zona fasciculata are seen tubules made up of small cells which have been pushed aside and evidently compressed. Such a condition is commonly seen in the normal gland just at the side of a small area undergoing fatty metamorphosis.

The most prominent departure from the typical structure, is seen in the region immediately adjacent to and including the upper medullary portion or in the zona reticularis (Plate XXVIII, Fig. 2). In specimens hardened in alcohol, there is an evident increase in the normal content of pigment, of a bright, brownish-yellow hue, both in the cell bodies and free in the intercellular substance. So great is the accumulation of pigment granules that sections stained with Delafield's hæmatoxylin and eosin-blue do not reveal the nuclei in this region. Potassium ferrocyanide and dilute hydrochloric acid fail to give evidence of iron in the pigment granules. The cells of the zona reticularis, instead of appearing in small anastomosing columns, remain more or less distinct in clusters of two or three. This appearance is to be interpreted as evidence of simple atrophy.

The intercellular spaces are large and prominent and appear as finely granular areas, in which delicate threads of the normal reticulum are distinct.

The polymorphism of the cells of the zona reticularis is very noticeable. They vary from one-third to twenty times the size of the typical cell in this situation. Many large homogeneous cells of giant size are seen (Plate XXVIII, Fig. 1). Each of these possesses a single large, deeply stained nucleus, usually about the centre of the cell. Many of these nuclei have a diameter ten times that of those in adjacent cells. The cytoplasm appears almost homogeneous, stains with eosin a delicate pink and, without definite limits, shades off insensibly into the surrounding intercellular substance. These I take to be hypertrophic cells undergoing necrobiosis. Many of the smaller cells in this zone also show evidences of degeneration and necrosis. Many are polychromatophilic with indistinct fragmented nuclei; others appear swollen, granular and devoid of nuclei, while in places the original cell is represented by nothing but a small mass of pigment.

In this region, as already mentioned, the intercellular substance and fibrous reticulum, are especially prominent, less apparently from actual new formation, than on account of the atrophy and disappearance of the columns of cells. Sections treated with picro-acid-fuchsin, fail to show any definite increase of the fibrous stroma. The reticulum is throughout rich in round and fusiform, deeply staining nuclei and especially so about the smaller capillaries.

The blood-vessels of the adrenals show in many places a pronounced perivascular infiltration with small round cells. The walls of the larger vessels present in areas peculiar nodular thickenings, not unlike those first described by Kussmaul, as periarteritis nodosa. There is a moderate nodular fibrous thickening of the intima of the vessels, but not so marked as to encroach materially upon the lumen.

The medullæ of both adrenals show but little microscopical evidence of disease. Here and there are seen, embedded in the medulla, portions of the cortex, a finding not uncommonly met in the normal organ. This portion of the gland is relatively small in amount, but present in all the sections near the hilum. As the edge of the gland is reached, it becomes narrower and ceases entirely about 1 cm. from the free edge. At the junction of the medulla with the cortex, I was able by the Nissl method to demonstrate here and there ganglion cells staining deeply and showing the same picture as that seen in cells of the semilunar ganglia.

Sympathetic ganglia: The right and left semilunar ganglia were hardened in absolute alcohol. Sections were stained by the Nissl stain, Van Gieson's method and hæmatoxylin and eosin. With simple stains the ganglion cells appeared numerous, without evidence of atrophy or degeneration. With the Nissl stain, the cells appeared large and fully formed, staining deeply and without evidence of chromolysis. At the borders of many of them were seen dark brownish metaplasm granules, usually appearing as a little irregular cluster on one side of the cell. The Nissl bodies were fully formed, standing out distinctly in the cytoplasm. A few of the ganglion cells contained two well-formed and distinct nuclei. This condition has been observed a few times before in different conditions, but is not believed to have any pathological significance. Nor to the increase of pigment can especial significance be attached. Otherwise no lesion is apparent. There was no small-celled infiltration, necrosis, caseation or any condition even suggestive of simple or tubercular inflammation in the cœliac ganglia.

The following facts in this case of Addison's disease seem to me worthy of note: the appearance of the cutaneous pigmentation fourteen years before the onset of the profound constitutional symptoms; the occurrence of severe rheumatoid pains in the extremities, so commonly observed among the early manifestations of the disease; the profound weakness with uncontrollable diarrhœa, epigastric pains and bilious vomiting; the prolonged duration of life (six months)

after the onset of the severe diarrhœa and vomiting; the irregular and atypical temperature; the absence of albumin or sugar from the urine; the characteristic color and distribution of the pigment; the rather severe anæmia with hypoleucocytosis.

The condition of the adrenal bodies found at autopsy was certainly surprising, the only gross abnormality being a simple atrophy of quite moderate degree. The microscopical evidences of this condition may be briefly summarized as follows: diminution in size and number of the glomeruli of the zona glomerulosa; diminution in length with great irregularity in the course and arrangement of the columns, as well as diminution of their fatty contents; colloid degeneration of circumscribed areas in the zona fasciculata; marked atrophy with increased pigmentation of the cells in the zona reticularis and replacement of some of the cells by clumps of pigment; general diminution in size of the medulla associated with the presence of displaced cortical structures; the presence of many mononuclear cells of gigantic size, of cells with fragmented nuclei and cells devoid of nuclei; thickening of the vessel walls with perivascular infiltration, and absence of chronic interstitial or tubercular inflammation.

These conditions warrant the diagnosis of *simple atrophy*, associated, perhaps, with attempts at compensatory cellular hypertrophy.

Assuming this condition, without lesion of the abdominal sympathetics, to be the essential cause of the clinical symptoms in this case, it may be well to call to mind other conditions concerned with the pathogenesis of this disease.

Addison, in his original memoir, considered that any lesion of the adrenal bodies, which interfered sufficiently with their function, could give rise to the disease.

Guy and Fowler, however, found that malignant lymphadenoma destroying the cœliac ganglia, without involvement of the adrenals, was capable of causing the classical symptoms. Rolleston, in the Goulstonian lectures for 1895, gives the following morbid conditions in the adrenals as possible etiological factors: Caseating tuberculosis; simple atrophy; chronic interstitial inflammation; malignant disease; occlusion of the adrenal vein; hæmorrhagic states. To these he adds destructive lesions of the semilunar ganglia.

From this classification, we are to believe that next to tubercular disease of the adrenal bodies, simple atrophy is the most common cause. Examination of the records shows reports of at least 16 cases of atrophy of the adrenals associated with symptoms of Addison's disease.

The cases reported by Osler,* Monti,† and Hadden,‡ respectively, should be classified as degenerative atrophies associated with chronic interstitial inflammation. There thus remain for consideration only 13 cases. Brief citations from the various records may not be out of place here:

Case I. (J. K. Spender, *Brit. Med. Journ.*, 1858.) Female, aged 53. Time of the first appearance of the pigmentation unknown. Attacks of profound prostration, epigastric pain, bilious vomiting and diarrhœa. Skin of entire body of a yellowish-brown color. At autopsy entire absence (?) of both adrenals.

Case II. (J. W. Legge, *St. Bartholomew's Hosp. Rep.*, 1874, X.) Female, aged 37. 18 months before admission noticed dark brownish streak on lip; 6 months ago, face became pigmented and there appeared weakness, shortness of breath and frequent attacks of bilious vomiting. Yellowish-brown color over entire body, especially over face, neck and dorsum of hand. Small patches of pigment on buccal mucous membrane. Symptoms became exaggerated and patient died one month after admission. At autopsy no evidence of tuberculosis in the body. The right adrenal absent but replaced by a mass of fat supplied by the adrenal artery and vein. The left adrenal thin, fibrous, without trace of the normal cortex. Microscopic examination revealed fully formed connective tissue, fat cells but no remnants of the gland. No coarse disease of nerves or ganglia.

Case III. (Goodhart, *Trans. Lond. Path. Soc.*, 1882.) Coal miner, aged 20. Admitted October 7, 1881, on account of increased weakness. Skin had begun to darken two years before. Pigmentation appeared first upon glans penis, then on face, arms, legs, etc. One year before admission attacks of severe epigastric pain with nausea. Skin dry, of a marked brown color, especially on sides of body, buccal mucosa, nipples, scrotum and glans penis. The diarrhœa, vomiting and weakness in-

* *Internat. Med. Mag.*, 1896-7, v, 3.
† *Arch. f. Kinderh.*, 1884-5, vi, 319.
‡ *Trans. Lond. Path. Soc.*, 1885, xxxvi, 436.

creased and the patient died in coma. Aside from the hyperplasia of the adenoid tissue of the gastro-intestinal tract, the principal post-mortem findings were in the adrenals. In the place of each of these bodies was a thin layer of a reddish-brown substance, still retaining the triangular outline of the gland and connected with the large adrenal vein. The glands were so thin, that, when placed between the fingers, they could hardly be appreciated. Microscopically the tissue showed simple atrophy without evidence of tubercular disease or implication of the cœliac ganglia.

Case IV. (Goodhart, *Trans. Lond. Path. Soc.*, 1882.) Draper, aged 44. For twelve years has had rheumatic pains in the knees. Eight years before skin became pigmented. Acute melancholia, necessitating confinement in a sanatorium, appeared for some months, but from this there was entire recovery. For a month before death, emaciation, weakness, nausea, vomiting and epigastric pains were manifested. At autopsy all organs healthy, except the adrenals, which were so wasted as to be scarcely visible. No evidence of suppuration or of caseous material. Apparently there was simple atrophy until each gland was reduced to the size of a split pea.

Case V. (Davy, *Trans. Lond. Path. Soc.*, 1882.) Machinist, aged 25. 3 years before entrance to hospital noticed pigmentation of nose, followed shortly by pigmentation under eyes. Gradually entire body became yellowish-brown. At times profound prostration. Death in coma following alcoholic intoxication. Entire surface of body uniformly pigmented, especially over genitals, flexor surfaces of joints and axillæ. Conjunctivæ pearly white, pupils minutely contracted. Left adrenal very much smaller than usual but of normal appearance except in one place near the middle. Right adrenal presented even less recognizable structure. Semilunar ganglia not found on account of fat.

Case VI. (R. G. Hebb, *Lancet*, 1883, I.) Female, aged 48. Pigmentation of skin noticed four weeks before, preceded by emaciation, attacks of headache, vertigo, nausea, vomiting and constipation. General pigmentation, especially over neck, elbows, areola of nipples and buccal mucosa. At autopsy no evidence of tuberculosis. Adrenals weighed 28 and 19 grains respectively, were of normal shape, of reddish color and soft, medulla apparently absent. No lesion of sympathetic ganglia observed.

Case VII. (Sidney Coupland, *Trans. Lond. Path. Soc.*, 1885.) Stonesawyer, aged 34, admitted June, 1881. Present ailment began two years ago with loss of appetite, vomiting, weakness and pigmentation of skin, which became deepest on face, dorsum of hands, neck and buccal

mucous membrane. Patient had many attacks as described and was under observation two and a half years. In January, 1884, following over-indulgence in alcohol, he succumbed, but was conscious until the last few hours. Mesenteric glands and Peyer's patches enlarged. No trace of right adrenal—possibly replaced by a small mass of fat. Left adrenal one-third normal size, of usual shape, very thin and almost translucent. Semilunar ganglia normal.

Case VIII. (Thomas Barlow, *Trans. Lond. Path. Soc.*, 1885.) Female, aged 42, admitted July, 1883. Pigmentation of skin began one year before; pregnant for last nine months; attacks of weakness and vomiting. On July 1, birth of a still-born child, followed by great prostration and death, in a typhoid state, a short time afterwards. At autopsy, pigmentation of entire body, deepest over areola of nipples, groins, dorsum of feet, under side of knees, etc. At first it was believed that the adrenal bodies had vanished without a trace, but careful dissection indicated the fibrous investment of the bodies, still preserving their cocked-hat shape and containing the merest trace of pale glandular tissue in each, which on microscopical examination gave evidence of the columnar arrangement of the cortex.

Case IX. (J. W. Legge, *Lancet*, 1885.) Female, aged 29, admitted May, 1884. Six months before had an attack of vertigo with vomiting. One month later noticed pigmentation of the skin, of a tawny brown color, deeper over right eye, xyphoid cartilage and axillæ. Clinical course characterized by fever, vomiting, progressive asthenia, deepening of the pigmentation and death. Autopsy: Lungs normal; right adrenal represented by a small shred of tissue about one-half inch long and one-quarter inch broad, left black, of natural outline, but completely wasted and thin as paper; solar plexus to naked eye normal.

Case X. (Senator, cited from Lewin, *Charité-Annalen*, 1885, p. 648.) Female, aged 53. For past six years attacks of vomiting; six weeks ago black masses of blood vomited, at same time high fever and epigastric pains. One month ago, small dark spot appeared on the gums. Soon entire body became bronzed, the pigmentation being especially marked upon the face. Many dark brown spots on face, arms and groins; skin, especially of fingers, rough and scaly; hair completely gray; iris yellowish-brown, sclera somewhat gray. Albuminuria with hyaline casts and pus corpuscles. Pulse 152. Adrenals markedly atrophic, the cortex being represented by scarcely more than two separate leaflets.

Case XI. (P. Guttmann, cited from Roloff, Ziegler's *Beiträge*, ix, p. 344.) Male, aged 20. One year before noticed pigmentation of the

skin, followed by increasing weakness, vomiting, epigastric pains, sopor and death. Autopsy: pronounced brownish pigmentation, most marked upon face, mucous membrane of mouth and lips, genitals, nates and bend of knees. Induration of pulmonary apices. Both adrenals reduced to mere traces of cortical substance. Cervical (? cœliac) ganglia normal.

Case XII. (Roloff, Ziegler's *Beiträge*, ix, p. 329.) Male, aged 22. Pigmentation, which increased in intensity, began 13 years previously, after an attack of acute pleurisy. Patient was comparatively well until five days before death. Constitutional symptoms began with loss of appetite and weakness. The day before his death, for the first time had vomiting and epigastric pains. He soon became delirious and died. Autopsy: acute hyperplasia of the gastro-intestinal lymph nodes; chronic pulmonary tuberculosis; right adrenal, 20x12x3 mm., markedly atrophic, left a little larger, but not more than $\frac{1}{4}$ to $\frac{1}{5}$ usual size, without evidence of tuberculosis in either gland, either macroscopically or microscopically. Microscopical examination revealed total absence of fat in the cortical cells. The principal portion of the gland remaining is the medulla. Here and there are small islets of tissue resembling the zona glomerulosa and zona reticularis. Microscopically semilunar ganglia normal.

Case XIII. (Bramwell, *Brit. Med. Jour.*, 1897.) Grocer, aged 37. Typical case of Addison's disease, characterized by profound and causeless asthenia, pigmentation of skin and buccal mucous membrane, pain in neck, occasional vomiting, slight emaciation. Death from influenza. Autopsy: lungs normal; complete absence of adrenals, their place being taken by masses of fat, which on the left side presented appearances suggestive of the remains of the degenerated adrenals. Microscopical examination revealed only a mass of fat cells and blood-vessels without trace of glandular structure.

It is seen that of the 14 cases reported (including my own), tuberculosis was present in the body only three times, being absent in all instances from the adrenals themselves. In none of the 13 cases from the records was the lesion of the glands relatively so slight as in the present case. The adrenals in case XII were apparently the largest of the series, and even the largest was less than 3 cm. in its greatest diameter. In my case they were nearly of normal size.

In two of the cases (I and XIII) the glands were apparently entirely absent, suggesting complete aplasia. Zander and others have noted

such a condition, but always, so far as I am aware, it was associated with defective embryological developments in other portions of the body, and it can hardly be supposed that the patients could have survived so long with actual aplasia of the adrenal glands. Case XII is at least suggestive of hypoplasia. Here the pigmentation of the skin began when the individual was nine years of age and continued for thirteen years, his health remaining fairly good until five days before death. The pathogenesis of this form of simple atrophy of the adrenal bodies is entirely unknown.

In attempting an explanation of this condition, the general causes of simple atrophy may be referred to: (1) pressure; (2) disuse; (3) senility; (4) neuropathic influences; (5) impaired nutrition. The first three need not be discussed. The possibility of defective inheritance may be suggested, but of its participation in the etiology of the disease we have no conclusive evidence.

In regard to neuropathic influences, there is evidence that lesions of the sympathetic may produce indirectly atrophy of the adrenal bodies, and as a result the symptom-complex of Addison's disease. Whether or not the ganglion cells have a trophic influence upon the cellular parenchyma of the adrenals, analogous to that of the anterior motor cells of the spinal cord upon voluntary muscular fibres, is not positively known, but recent physiological experiments fail to strengthen this supposition. von Kahlden and others have shown that a lesion involving the splanchnic nerves without direct lesion of the semilunar ganglia, may produce these clinical symptoms, and Biedl has shown that the secretion of epinephrin is under the influence of these nerves. In our case the splanchnic nerves were not carefully dissected out and examined, but it is certain that no gross or histological changes were present in the cœliac ganglia.

It has been suggested that certain cells in the spinal cord may exercise a trophic influence upon the adrenal parenchyma, but there is only negative evidence to support this hypothesis. The brain and cord of the present case were not obtained for microscopic examination.

Defective nutrition cannot be excluded as a possible causative factor in this case, but no such changes were observed in the adrenal vessels as would indicate serious disturbances in the local circulation.

It must be admitted, therefore, that at present we are as much at a loss to understand the pathogenesis of this condition as was Spender in 1858.

The work of Oliver and Schaefer,* and more recently that of Abel,† has shed light upon the function of the adrenals. The blood-pressure-raising constituent, designated by Abel "epinephrin," has been shown to be derived from the medulla, but the exact rôle that it plays in normal metabolism or in the production of the pigmentation and other phenomena of Addison's disease has not been demonstrated. The medulla in the glands of the case just reported, though as a whole somewhat diminished, is nevertheless present in comparatively fair amount.

It is difficult to believe that the symptom-complex of Addison's disease can be explained by such quantitatively insignificant histological changes as were present in this case. It is well known that certain glandular organs may undergo perversion of function and still present little or no histological evidence of disease. There is evidence that some cases of diabetes mellitus are referable to disordered function of the pancreas without manifest histological lesions of this organ. Recent investigations have demonstrated the vital importance of the adrenal glands and their endowment with a so-called internal secretion. If it were possible to estimate clinically the amount of epinephrin secreted, as we estimate the amount of urea in the urine, we might then be in a better position to explain such obscure case of Addison's disease as the one reported in this paper. Although Addison's disease is undoubtedly due to some interference with the function of the adrenal bodies, it seems probable that, in this case at least, we have to deal with a perversion rather than with a total lack of function.

DESCRIPTION OF PLATE XXVIII.

Fig. 1. From section of adrenal. *A*, large cell with vacuoles; *B*, cells of the zona reticularis; *C*, perivascular infiltration. Zeiss ocular 2, objective D.

Fig. 2. Section of adrenal. *A*, portion of normal zona fasciculata; *B*, simple atrophic cells of the zona reticularis; *C*, atrophic and hypertrophic

* Oliver and Schaefer, *Journal of Physiology*, xvi and xviii.

† Abel, *Bull. of the Johns Hopkins Hospital*, 1897, p. 153, and *Zeitschr. f. physiol. Chemie*, xxviii.

cells in contiguity; *D*, pigmented protoplasmic remains of cell bodies; *E*, connective tissue reticulum prominent on account of degeneration and disappearance of the parenchyma; *F*, normal cells in zona reticularis.

The red masses seen in some of the cells correspond in reaction to the colloid masses described as occurring in the zona fasciculata.

Technique. Hardened in Müller's fluid, stained in Delafield's hæmatoxylin and one per cent aqueous solution of eosin-blue. Zeiss ocular 2, objective D.

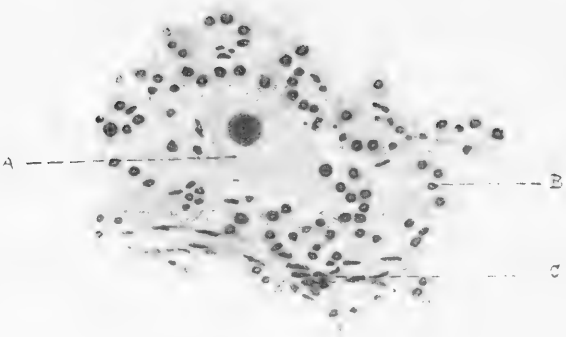

FIG. 1.

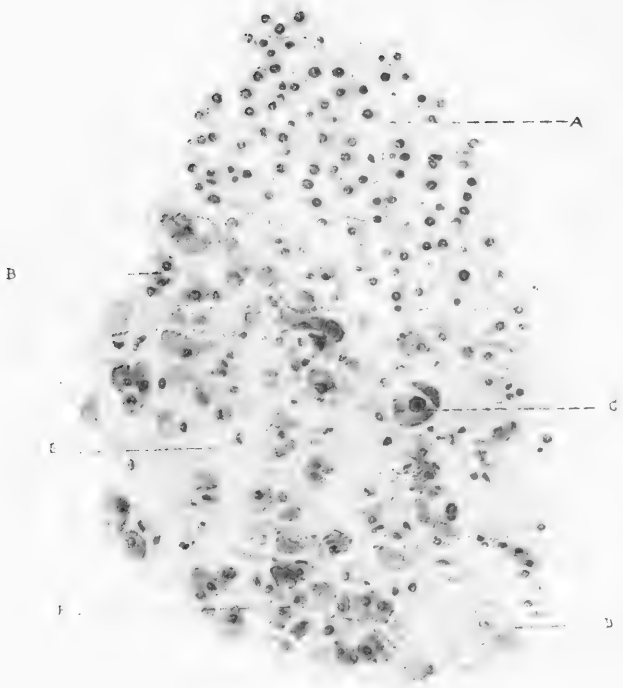

FIG. 2.

A PECULIAR FORM OF FIBROSARCOMA OF THE BRAIN.

By ALICE HAMILTON, M. D.

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PLATES XXIX AND XXX.

Fibrosarcoma of the brain, though described with relative frequency by earlier writers, scarcely finds mention in later text-books of pathology, and though individual cases are still recorded in the current literature, they almost invariably come from observers who base their diagnosis chiefly upon the gross appearances, and whose description of the microscopic findings is far from satisfactory. A careful examination of the literature on the subject forces the conviction that the majority of tumors which have been classed as fibrosarcomata were in reality gliomata unusually rich in fibres. Indeed, the tendency of pathologists now is to regard as gliomata all tumors of the nervous system, which cannot be proven to have taken their origin either in the meninges or in the walls of the blood-vessels, in the latter case usually in the endothelium. It is, therefore, with some hesitation that I venture to give the name of fibrosarcoma to a primary growth in the cerebrum, a growth which cannot have originated in the meninges nor in the endothelium of the vessels. It seems, however, impossible to class it otherwise than as a connective-tissue tumor of a very peculiar type, originating probably in the outer coats of the blood-vessels.

The specimen was given me for examination by Dr. L. L. Skelton, of Chicago, to whom I am indebted for the following brief notes of the clinical history and autopsy. The patient was a married woman, twenty years of age. When seen by Dr. Skelton in consultation she was suffering from "terrific" headaches, and gradual impairment of vision. There were slight incoördination, hysteria, psychic changes

and intermittent albuminuria. Ophthalmoscopic examination showed choked disks. Dr. Skelton diagnosed a tumor of the brain, and advised operation. This was refused, and he did not see the patient again until after her death, when he was requested by the family physician to make an autopsy. This was done twenty-four hours after death. Upon removing the skull-cap the right cerebral hemisphere was seen to bulge a little, but the meninges and cortex were apparently unaltered. When, however, a shallow incision was made in the frontal region through the cortex, the latter peeled away, leaving exposed a spherical mass distinctly circumscribed and readily enucleated. This mass occupied the deeper parts of the three frontal convolutions and subjacent white substance projecting into the ventricle, but not involving the basal ganglia. No important changes were found in the thoracic or abdominal viscera; there were no new growths in any organ save the brain.

The specimen, when it came into my hands, had been for eighteen months in a four-per-cent solution of formaldehyde. Unfortunately the brain had not been preserved in toto, but the tumor had been removed with only that part of the tissues which directly surrounded it, so that the exact relation of tumor to brain could not be determined. The mass was about as large as a medium-sized orange and firm in consistence; its color was whitish with gray streaks. The cerebral tissue separated easily from the tumor; in no place was there any sign of infiltration, extension of the growth having evidently taken place by displacement of the normal tissue. Pieces from different parts of the tumor were hardened, some in alcohol, others in potassium-bichromate-chromalum solution according to Weigert's quick method for myelin sheaths, and others in picric acid and ammonium bichromate for Mallory's neuroglia stains. Sections were stained in the above-mentioned ways, and also with hæmatoxylin and eosin, with Van Gieson's picro-acid-fuchsin, and with Weigert's new fuchsin stain for elastic fibres.

The first sections examined were those which had been cut from the edges of the tumor, where the new growth seemed, to the naked eye, to be sharply divided from the uninvaded brain tissue; this im-

pression was confirmed under the microscope. The tumor was circumscribed, although not encapsulated, and there was no sign of a gradual infiltration of surrounding parts. Furthermore, the meninges were found to be uninvolved in the new growth. Often the normal tissue was separated from the tumor mass by a prolongation of the pia mater in between two convolutions, while the membrane itself was normal save for slight round-cell infiltration.

Examined under the low power of the microscope, the tissue of the tumor was seen to be exceedingly rich in cells, closely crowded together, and with very scanty intercellular substance. The cells were not evenly distributed, being often gathered together in clumps, which seemed composed of more or less circular elements, while bands of spindle-shaped cells occasionally ran across the field between the clumps. Spindle cells could also be seen near the blood-vessels, and these cells were possessed of processes, while the circular and oval cells were apparently devoid of processes. The intercellular substance had usually a granular appearance, but between the spindle cells it was delicately fibrillar. Under the high power the cells exhibited a great variety of shapes and sizes. So closely were they packed together in some places that it was impossible to distinguish their individual outlines, the appearance being that of a large mass of protoplasm with nuclei scattered through it. In thinner places, however, the cells stood out clearly, the protoplasm of the largest ones being granular, and taking the stain deeply. Mallory's phosphomolybdate stain brought out the cells most distinctly, and sketches were made of the different types in sections stained after this method (Plate XXIX, Fig. 1). The cell which seemed most widely distributed was the round, or irregularly oval, or angular cell of medium size, with lightly stained protoplasm and a single nucleus, the latter usually circular, staining deeply at the periphery and showing coarse chromatin granules. Apparently naked nuclei, singly or in clumps, occurred with great frequency, and occasionally very small cellular elements were found with a pyriform body and one delicate process. The spindle cells, mentioned above, occurred, not scattered among the other cells, but in slender bands or close to the blood-vessels. They

possessed but one nucleus, and one or more processes at each end. Some of them resembled the so-called brush cells. They lay with their long axes parallel, and their processes formed a fibrillar matrix, which was wanting elsewhere in the tumor. The larger cells were most irregular in outline and in the arrangement of their nuclei, which were sometimes as many as ten in number. The protoplasm was more granular than that of the other cells, and stained deeply; the nuclei were richer in chromatin. These cells were often found in groups, but no part of the tissue was totally devoid of them. They were frequently vacuolated, and this gave rise to appearances like those interpreted by some observers as intracellular parasites. Sometimes these cells would have one delicate process, rarely more, but the majority were altogether devoid of processes.

As can readily be seen from the foregoing description, the cellular elements demonstrated in this tumor are not those usually found in glioma, but suggest a connective-tissue growth. Gliomata containing irregularly shaped giant cells have been described by Fleischl,* by Klebs, by Stroebe, and by myself, but in all these cases the majority of the cells in the tumor were either spider, brush, or the so-called ganglion cells, all of which were furnished with processes, and the intercellular substance was rich in fibres. An attempt to stain sections of this tumor by Golgi's method failed entirely, but this may have been due to the length of time which elapsed before the autopsy was made, and perhaps also to the long immersion in formaldehyde.

The tumor was unusually rich in blood-vessels of all sizes, from capillaries to large vessels with several layers of elastic and muscular fibres in their coats. The smaller vessels presented no peculiarity in appearance, but in the larger ones the outermost fibres, which composed the wall, could often be seen passing out into the tissue to lose themselves among the cells at a long distance from their origin (Plate XXX, Fig. 4). These fibres had all the characteristics of elastic tissue. They pursued a wavy course, were curled at the free ends, and did not branch. Again, in other places, fibres exactly like these in character were found running between the cells, though they could

* References to literature are at the end of this article.

not be traced to any vessel, and had no apparent connection with the cells of the tumor. By Van Gieson's stain they came out in sharp contrast to the cells, staining pink or deep red (Plate XXIX, Fig. 2); by Mallory's phosphotungstate they stained a purple red (Plate XXX, Fig. 3); but it was in sections treated with Weigert's fuchsin stain for elastic fibres that they could most easily be traced (Plate XXX, Fig. 4). By this stain they appeared bluish-black, and therefore quite different from the delicate intercellular fibrils and processes from the spindle cells, which by all of the above-mentioned methods took the usual protoplasmic stain. The staining properties of these fibres forbade the supposition that they could be remains of medullated nerves. Sections were stained by Weigert's differential method for myelin sheaths, with the result that the medullated nerves were found to end abruptly at the edge of the tumor, no trace of such a structure being demonstrable within the new tissue. This absence of medullated nerves is regarded by Stroebe as one proof that a tumor is not of nervous origin.

Much more striking, however, than the single scattered fibres, were the thick circumscribed collections of coarse and delicate fibres which appeared everywhere throughout the tumor. These were dense masses lying among and on top of the cells, being sometimes long and spreading, sometimes round. They appeared in every variety of shape, from symmetrical rosettes to long irregularly branching masses. The fibres which composed them were usually delicate, and curved slightly, sometimes thick and bristling. In the larger masses, and in almost all the circular ones, the centre was composed of a granular material, the fibres appearing clearly only at some distance from the centre (Plate XXX, Fig. 3). Under the low power it often looked as if the fibres were processes of the surrounding cells, and passed from them into the centre, an arrangement suggesting that of the tails of the spermatozoa in a seminiferous tubule of the testicle. A close examination, however, under the oil immersion failed to show that these fibres were connected with the cell bodies; they seemed simply to pass among and over the cells. The cells directly surrounding them were usually spindle-shaped, sometimes with, sometimes

without processes. When present, such processes took the protoplasmic stain.

Although no part of the tumor was free from these masses, yet it was noticeable that the larger the number of blood-vessels, the fewer the fibrillar masses. Many of them occurred in close connection with the vessel walls, either entirely surrounding a small vessel, or covering one side, or, what was most common, scattered at intervals along the course of a larger vessel. Occasionally a rosette would contain in its centre a small collection of cells (Plate XXIX, Fig. 2).

A close connection between the fibrillar masses and the vessel walls suggested the idea that this connection might be invariable, and that in cases where the masses seemed independent, they were lying along the wall of a vessel which had not been included in the section. To prove this, serial sections were made, but with the result that by far the larger number of these masses were without demonstrable connection with the vessels.

In their staining properties these fibrillar masses corresponded with the single scattered fibres already described, and thus produced a very striking appearance, especially in sections stained by Van Gieson's method, where the deep pink rosettes stood out in sharp contrast with the yellowish background. By Weigert's elastic-fibre stain it was often possible to follow the single scattered fibres and see them end in a rosette, and also in many cases to find such fibres running from the wall of the vessel to lose themselves in rosettes at varying distances from the vessel (Plate XXX, Fig. 4). Though the fibrils composing the rosettes were usually much more delicate than the diffuse fibres, and stained a little lighter, yet there seemed no room for doubt that the two were of the same origin and character.

I can find in the literature no description of collections of fibres such as these in the case under discussion. Gliomata rich in fibrils are of frequent occurrence, but in these the fibres are never arranged in circumscribed masses, nor do they take the differential stains for connective tissue. By the kindness of Dr. Flexner I was enabled to compare with this tumor sections from an ependymal-celled glioma described by him a year ago. The arrangement of the fibres in his case on

first sight resembled somewhat the arrangement in the one I am describing. Here, too, there are long and circular collections of fibres surrounded by cells, but these fibres are processes from the cells from which they pass to the vessel walls; they stain as do ordinary glia fibres, and the cells are of the type of ependymal cells. The two specimens cannot, therefore, be regarded as belonging to the same class of growths.

The question then resolves itself into the following: Is this a glioma with a peculiar form of sclerosis, or a fibrosarcoma with masses of elastic fibres in the matrix? There are several arguments in favor of the former alternative. In the first place, it is unusual to find a connective-tissue growth in the cerebrum which has not sprung from the meninges, or from the endothelium of the vessels. The possibility that the connective-tissue elements in the outer and middle coats of the vessels might proliferate and form a true sarcoma cannot be denied, but this is not the usual form of connective-tissue growths in the nervous system. Upon the assumption that the tumor is a glioma, the apparent dissociation of fibres and cells might be explained by considering that this tumor represents the adult stage of neuroglia, when, according to Weigert, the fibres no longer have connection with the cells. Taylor argues in favor of this view, and has given a description of two specimens of glioma, the one representing the embryonic stage, the other the adult stage of neuroglia. In the former the fibres of the matrix proceed from the cell bodies; in the latter they are entirely independent of the small round cells. Maximilian Herzog also follows Weigert's view as to the dissociation of glia cells and fibres, but modifies it by regarding the fibres as cells which have undergone a senile change, analogous to the cornification of epithelial cells. He described an ependymal cyst lined with cubical epithelial cells. The cells became slender bipolar spindles as they approached the edge of the cyst, refractive granules appeared in the protoplasm, and finally the free edge was covered with stiff slender fibres, staining like cornified material. He argues that in this case the neuroglia cells, being of epithelial origin, reverted to the original type and formed a true epithelial growth, but that the process here

was in all essentials analogous to the formation of the fibres of neuroglia by gradual flattening and cornification of the cells. He might, therefore, consider the collections of stiff fibres in my case as analogous to the cornification of the cells in the centres of epithelial pearls in carcinomatous growths.

On the other hand, the arguments in favor of the connective-tissue nature of the tumor are many. In gross appearance it differs from a typical glioma, for it is circumscribed, firm in consistence and easily enucleated, while a glioma usually infiltrates instead of displacing the normal tissue, and is soft in consistence. Again, the characters of the cells and of the intercellular substance do not suggest a growth springing from the neuroglia. The entire absence of medullated fibres is another point against glioma.

The strongest argument, however, in favor of the connective-tissue origin of the growth is based on the chemical nature of the fibres, as shown by their reaction to differential stains. The distinctive chemical characteristics of neuroglia fibres, as evinced by their affinity for certain stains, is especially emphasized by Weigert in his exhaustive work on human neuroglia, and is made by him the basis for determination as to what does and what does not belong to this tissue. It is much to be regretted that the tumor was not obtained in the fresh condition, so that it could have been stained by Weigert's method for neuroglia fibres, but in the absence of this test the reaction of the fibres to the three stains mentioned above (Van Gieson's, Mallory's phosphotungstate, Weigert's elastic-fibre stain) would seem to prove conclusively that they belong to the connective tissues. The origin of the growth must be from the connective-tissue elements in the walls of the blood-vessels, and these elements, instead of producing a fibrosarcoma of the usual type, have gone on to the production of polymorphous cells with aggregations of elastic fibres within the matrix. That this is unusual must be conceded, but there is certainly an analogue to such a process in the formation of islands of hyaline cartilage in chondrosarcoma, and of bone in osteosarcoma.

I am unable to find descriptions of fibrosarcomata in which the fibres of the matrix were proven to be elastic fibres. It would seem that the

question as to the exact nature of such intercellular fibres in these tumors has not been systematically studied. The application of Weigert's admirable stain for elastic fibres to the study of tumors and of other pathological conditions gives promise of yielding interesting results. Melnikow-Raswedenkow in a recent article has described the distribution of elastic fibres in various organs in normal and pathological conditions. He notes especially the richness in elastic fibres of the walls of blood-vessels, particularly of the adventitia, and finds that the elastic fibres in healthy and diseased organs and tissues are derived mainly from the vascular walls. Within the central nervous system elastic tissue is scanty and present only in the walls of blood-vessels. He states that in tumors no new formation of elastic tissue occurs, a statement, however, contradicted by the presence of a large amount of elastic tissue in the tumor now under consideration. There is no other apparent source for the elastic tissue found in the present tumor than that in the walls, especially the adventitia, of the blood-vessels, and this conclusion is in accord with Melnikow-Raswedenkow's observations concerning the normal distribution of this tissue in the brain as well as with the relationship, already described, of much of the elastic tissue to the vessel walls. Where this relationship is no longer apparent, its disappearance may be attributable to obliteration of vessels or to the severance of the original connections.

The literature on the normal development of elastic fibres is most unsatisfactory. There seem to be two theories as to the origin of these fibres—the cellular and the intercellular theory—dating from Theodor Schwann's belief in the transformation of the cells into fibres and Max Schultze's view that the cells fused to form the fibrillæ. B. Lwoff believes that the fibres are formed by the outer part of the protoplasm of the cells, which becomes differentiated and lies as a sheath around the inner protoplasm and nucleus.

The later observers, almost without exception, hold that the fibrils are formed in the intercellular matrix, independently of the cells, or at any rate without visible connection with them. Henle, Kölliker, Ranvier, Schaefer, and Minot uphold this view. According to Ranvier, there appear in the gelatinous matrix between the embryonic

connective-tissue cells globules of elastin, probably deposited by the cells. These fuse and form fibrils. They appear late in embryonic life (fifth month in human beings), grow by thickening, and continue to form even after birth. According to Minot, the elastic fibres in the omentum develop thus: The connective-tissue cells become long and spindle-shaped, with oval nuclei. In between them appear fibrils which increase in length and number, and gradually form bundles, which take a wavy course. Throughout their development they have no apparent connection with the cells. Schaefer has observed that in the development of cœlenterates fibres appear in the matrix at a period when there is entire absence of cellular elements.

As regards the mode of development of the elastic fibres in the outer coats of the blood-vessels, the literature is even more scanty. The description of the formation of new blood-vessels may be followed with precision until we pass from the capillary tube with its simple endothelial coat to the arteriole with a fibro-muscular coat, where the subject suddenly becomes obscure. Most works on embryology ignore completely the question of the origin of these fibres. Minot says they are formed by the differentiation of the surrounding mesenchyma, while the pathologists seem to consider that, so far as concerns the newly-formed vessels in proliferating tissues, the outer coats are formed by multiplication and differentiation of the endothelium of the capillary walls.

So long as the whole subject of the normal development of elastic fibres is unsettled, it would be useless to attempt to explain their occurrence in a pathological growth. Whether, however, they are formed from the bodies of cells, or deposited by the cells in the matrix, or arise independently, it is certainly conceivable that a growth, originating in the connective-tissue elements of the vessel walls, might produce, in an abnormal and irregular way, the same kinds of fibres as its prototypes have produced in the course of normal development. As said above, the process would seem to be somewhat analogous to the formation of islands of cartilage or bone in mixed sarcomata.

In the tumor under discussion the steps in the process of fibre formation seem to be similar to those cited above as described by

Minot in the normal development of elastic fibres in the omentum. Spindle cells, like those which he describes as appearing before the fibres are found, occur in bands through the tissue of the tumor and, though furnished with processes, have no apparent connection with the real elastic fibres which are found between them. They would seem, however, to be in some way concerned in the formation of these fibres as they are present in greatest numbers in the neighborhood of the rosettes and the masses of fibrils which lie along the vessel walls.

DESCRIPTION OF PLATES XXIX AND XXX.

PLATE XXIX.

Fig. 1. Isolated tumor cells. Small, round and oval mononuclear cells. Small and large pyriform and fusiform cells. Giant cells with and without processes. One giant cell shows cellular inclusion. Leitz objective $\frac{1}{12}$ in. oil immersion, ocular 5. Mallory's phosphomolybdic acid hæmatoxylin.

Fig. 2. Typical field containing fibrillar masses of different shapes. One mass surrounds a small vessel almost completely. Two masses lie along the sides of vessels. One contains a group of cells in its centre. In one place scattered fibres pass between the tumor cells. Leitz objective $\frac{2}{3}$ in., ocular 3. Van Gieson's picro-acid-fuchsin.

PLATE XXX.

Fig. 3. One medium-sized rosette. The fibres can be seen passing over the cells. Large giant cells form a group to one side. Leitz objective $\frac{1}{12}$ in., oil immersion, ocular 5. Mallory's phosphotungstic-acid hæmatoxylin.

Fig. 4. Two small rosettes near a blood-vessel. Elastic fibres can be seen passing among the tumor cells to the rosettes, also from the vessel walls to the rosettes. Most of the cells are large, round and oval, but at one side can be seen a band of spindle cells. Leitz objective $\frac{1}{12}$ in., oil immersion, ocular 3. Weigert's elastic-fibre stain. Nuclei stained with lithium carmine.

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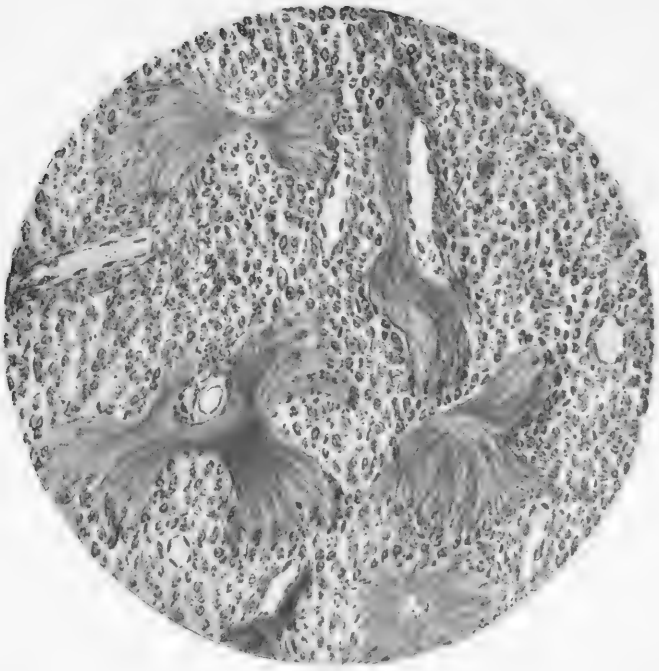

FIG. 2.

FIG. 1.

FIG. 3.

FIG. 4

ON THE DIFFERENTIATION AND CLASSIFICATION OF WATER BACTERIA.

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When bacteriologists have before them the problem of differentiation and classification of bacteria from water, independent of efforts to isolate with minimum delay some specific organism of disease, they proceed in a manner which is substantially as follows:

1. There is obtained on a given laboratory medium a pure culture of a special bacterium, the environment and life history of which, prior to its isolation at the laboratory, are for the most part unknown.

2. From the pure culture there are seeded a greater or less number of conventional culture media, known to differ in composition somewhat as prepared at different times at the same laboratory, and considerably as prepared by various workers in different laboratories.

3. These cultures are subjected at the laboratory to varying temperatures and periods of development leading up to final descriptions of cultural characteristics.

4. Observations upon and descriptions of the results obtained from the growth of this special bacterium under the above stated conditions are made.

5. Upon a comparison of the records of this bacterium with the limited descriptions of more or less similar forms given by other observers, it is found that they do not coincide, and therefore a new species is recorded.

Practically speaking, the above outline shows briefly and in a general way the conventional custom of bacteriologists in the past fifteen years, and which, as is well known, has brought the subject of species differentiation to a position which is untenable. Owing to the wide recognition of this state of affairs it seems to the writers to be futile to speak farther of the past.

It is the purpose of this paper to refer briefly to a number of points

associated with the above outlined procedure, and call attention to several modifications which experience, in a somewhat extended study of this subject, has led us to believe are of material assistance in the differentiation and classification of water bacteria.

I.—DEBILITATING ENVIRONMENT OF WATER BACTERIA BEFORE ISOLATION, AND THE CONSEQUENT DEGENERATION IN MANY INSTANCES AS NOTED AT THE LABORATORY.

Experience shows that in many cases two members of the same species of water bacterium, isolated at the same time and from the same sample, owing to divergent conditions in their earlier environments and in their life histories, will differ in some specific functions or characters, as studied in the laboratory. We have learned that, by transplanting successively such forms upon a series of media, these differences can, in a great measure, be eliminated. Accordingly, we have adopted the procedure of transferring the organisms after isolation from the pure culture on agar to nutrient broth; from the latter after three days' incubation at 20° C. to gelatine plates; and from a gelatine plate after the same period of incubation back to an agar tube again, from which the conventional media are seeded after the customary three days have elapsed. The value of this course, calculated to lend to the more or less debilitated form a rejuvenating action, and to bring the two members into a more nearly parallel physical condition before diagnostic tests are begun, is illustrated by the representative results given in Table I.

TABLE I.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Name of organism.	Test.	Result of Test.	
		Primary culture.	After preliminary cultivation.
Bacillus fluorescens liquefaciens.	Motility.	Negative.	Positive.
" " "	Nitrate reduction.	"	"
" prodigiosus.	Gas production.	"	"
" coli communis.	Coagulation of milk.	"	"
" ochraceus.	" "	"	"
" similtyphus.	Nitrate reduction.	"	"
" subtilis.	Motility.	"	"
" viridis.	Indol production.	"	"

In each case the temperature and period of development were 20° C. and 10 days, respectively, with the exception of the tests for motility, which feature was observed in cultures two days old.

Attention is particularly called to the last step in the process of preliminary cultivation, as it is important that, once having gotten an organism into a condition from which more favorable results may be obtained, the culture should be allowed to lose none of its regained vitality by being allowed to remain an unnecessarily long time on the agar tube before the various media are inoculated.

If the results of diagnostic tests are to be regarded seriously, and if

TABLE II.

EFFECT OF PRELIMINARY CULTIVATION ON BACTERIAL DEVELOPMENT.

Test.		Percentage constancy of results.	
		Primary cultures.	After preliminary cultivation.
Bacillus—true form,		95	100
Motility,		87	100
Spores—heat test,		93	100
Growth at 37° C.,		98	100
Liquefaction of	Gelatine,	98	100
	Casein,	95	100
	Blood serum,	93	100
Fermentation of dextrose broth	Gas,	92	100
	Turbidity in closed arm,	89	100
Nitrate reduction,		90	100
Indol production,		97	100
Milk coagulated,		80	100
Fluorescence,		95	100
Chromogenesis,		100	100

such results are to lead to classifications which shall be of permanent value, it is obvious that such results must yield a high percentage of constancy. Otherwise they will be misleading and must be eventually discarded.

For the purpose of determining the percentage constancy of the results of prominent diagnostic tests, before and after transplanting the culture upon a series of media as described above, twenty cultures, representing eleven different species of water bacteria, were carried through a series of tests, the results of which appear in Table II.

Each of the media was seeded in triplicate with each culture from which both morphological and biological results were obtained. Development was continued for 10 days at 20° C. before the final results of the respective tests were recorded, although observations were taken day by day.

II.—ON THE COMPOSITION AND PREPARATION OF CULTURE MEDIA WITH ESPECIAL REFERENCE TO LIMITATIONS IN THE USE OF NUTRIENT CARBOHYDRATE SOLUTIONS AS PREPARED AT PRESENT.

In this connection the recommendations of the Bacteriological Committee* leave but little to be desired, so far as is practicable to be obtained at present. And in our species work during the past ten months, it has been our custom in the preparation of media to follow the procedures recommended, with no departure except a limitation in the use of the different carbohydrate solutions as now prepared.

In regard to the use of carbohydrate solutions prepared by present methods, our experience has shown an inconsistency in quantitative results in connection with gas production and the end reaction. In quite an extended study of the accuracy of these tests, the results obtained show the influence of three factors, intimately connected with the inconstant results that were often obtained, which are as follows:

1. The results of growth in a carbohydrate solution are affected somewhat by the possible degeneration of the organism.

2. The results of growth are often affected by the initial reaction of the carbohydrate solution.

3. Irregularities in the composition of the carbohydrate solution as prepared and sterilized, notably in the case of lactose and saccharose, also affect these results.

An illustration of the first factor is presented below in Table III showing representative results upon the quantity of gas and the end reaction in dextrose, lactose and saccharose broths, by six different cultures of Bacillus coli communis isolated on successive days from

* Procedures Recommended for the Study of Bacteria, *Journal of American Public Health Association*, Jan., 1898.

the Ohio River water. The temperature and period of incubation in all cases were 20° C., and ten days, respectively; and the cultures were not subjected to preliminary cultivation.

TABLE III.

REPRESENTATIVE RESULTS OF GROWTHS OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS.

Number of organism.	Carbohydrate.	Initial reaction 1.5 per cent Acid.	
		Total gas (per cent).	End reaction (per cent).
1	Dextrose.	77	3.8
2		83	4.1
3		52	3.2
4		41	2.9
5		88	5.0
6		90	4.9
1	Lactose.	91	3.8
2		78	3.6
3		49	5.1
4		80	4.2
5		60	2.6
6		53	3.8
1	Saccharose.	48	3.0
2		0	2.7
3		13	1.9
4		15	0.8
5		2	1.0
6		2	1.6

From what has been stated with regard to the influence of preliminary cultivation, to overcome initial degeneration, as outlined in the last section, it would naturally be expected that such a procedure would eliminate in a measure the discrepancies in the above results. As shown in Table IV this preliminary step as carried out was appreciably helpful, but was incapable within the limits studied of making the quantity of gas of decisive diagnostic value.

Relative to the influence of preliminary cultivation upon the end reaction, it was found to be inadequate to bring about even fairly constant results, as shown in Table IV.

With regard to the second of the above factors it was found that within a fairly close range of initial reactions, as ordinarily employed and reaching from about + 1.5 to —1.5 per cent, its influence upon

TABLE IV.

GROWTH OF B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS, BEFORE AND AFTER PRELIMINARY CULTIVATION.

No. of Organism.	Carbohydrate.	INITIAL REACTIONS.											
		1.5 per cent. Acid.				Neutral.				1.5 per cent. Alkaline.			
		Before.		After.		Before.		After.		Before.		After.	
		Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).	Total gas (per cent).	End reaction (per cent).
A	Dextrose	32	3.9	68	4.2	43	3.8	57	2.7	52	4.2	68	3.3
B	Dextrose	46	2.7	59	3.7	37	2.9	62	3.8	40	4.4	72	3.9
C	Dextrose	59	3.1	81	5.1	70	4.2	54	4.0	26	5.0	56	5.0
A	Lactose	62	4.2	62	2.9	39	3.4	52	3.3	47	2.9	62	2.9
B	Lactose	27	5.1	48	3.8	64	4.1	69	3.7	38	3.6	51	3.6
C	Lactose	82	3.7	74	3.9	58	3.8	69	4.1	59	2.7	63	3.9
A	Saccharose	10	1.9	83	4.7	12	3.3	64	3.8	0	—1.4	30	2.6
B	Saccharose	0	1.4	4	2.1	0	0.9	1	0.7	0	—1.2	0	—1.4
C	Saccharose	0	1.6	1	1.7	0	0.6	1	0.6	0	—1.1	0	—1.4

the total amount of gas produced and the end reaction does not appear to be very marked. This is especially true if the period of incubation at 20° C. does not exceed 3 or 4 days. When the period of incubation reaches 10 days, then, even within the above stated limits, the initial reaction becomes a factor with reference to the final quantitative results.

But if the initial reaction should be more than about 1.5 per cent from the phenolphthalein neutral point, considerable variations in the results are found, as compared with those obtained with initial reactions within the stated limits. And in most cases it was noticeable that the quantity of gas was greatest in those tubes where the solution was initially most alkaline (—2.0 per cent). This is shown by the results presented in Table V (averages of more than 100 sets of results) with reference to B. coli communis freshly isolated from feces; and when the period and temperature of incubation were 10 days and 20° C. respectively.

TABLE V.

PERCENTAGE OF TOTAL GAS PRODUCTION BY B. COLI COMMUNIS IN CARBOHYDRATE SOLUTIONS OF DIFFERENT INITIAL REACTIONS.

Initial reaction (per cent).	Carbohydrate.	
	Dextrose.	Lactose.
+2.0	35	28
+1.0	36	34
.0	45	45
—1.0	53	52
—2.0	68	64

In connection with the third factor mentioned at the outset of this section, it will be noted in the foregoing tables that the fermentation data from saccharose solutions, and to a certain degree from lactose solutions, are variable to the point of being erratic. Numerous experiments show them, however, to be representative even when obtained with the same culture. That is to say, there is decisive evidence to show that the variable results were due to the culture solutions and not to the bacteria themselves.

To explain fully these variations involves a thorough knowledge of the chemistry of carbohydrates, and is beyond the scope of this paper. It is sufficient to state that the explanation, in part at least,

appears to be associated with chemical changes (inversion, etc.) which are produced directly by the action of heat. Special efforts for several months were made to obviate the disturbing influence of heat, by using intermittent sterilization of the solution at low temperature, 70° C.; the use of sugars sterilized by dry heat at low temperature for long periods; and by means of adding the pure sugar dissolved in sterile water to the broth after the sterilization of the latter. None of these efforts were successful, and other work caused an abandonment of these studies.

Summing up in brief terms the experience recorded in this section, it may be stated that the quantity of gas formed in carbohydrate solutions and the end reaction of the solution are found to be too indefinite to be of value in the classification of bacteria; and that by the aid of dextrose alone better (more decisive) qualitative evidence is obtained than by the use of dextrose, lactose and saccharose.

III.—THE TEMPERATURE AND PERIOD OF DEVELOPMENT OF WATER BACTERIA DURING CULTIVATION FOR CLASSIFICATION TESTS.

Temperature of Development.—It is undoubtedly true that, for the purposes of prompt differentiation of bacteria intimately associated with the causation of disease, and for detailed comparative studies of races or varieties of a given species, it is necessary that there shall be made comparisons of the results of growths of cultures at substantially 20° and 37° C.; yet, for the classification of water bacteria, it is our experience that cultivation at 20° for each set of tests is sufficient. Of course, it must be learned whether or not the bacterium will grow at 37° C.; and this, according to our present custom, is obtained by means of an agar plate culture.

In explanation of our departure from the custom of employing both 20° and 37° C. for temperatures of development, it is to be stated that such was our practice, in strict accord with the recommendations of the Bacteriological Committee, during the early part of our work at this laboratory. But soon we became satisfied that, for the purposes of classification of water bacteria, the increase in amount of definite information obtained from cultures grown at 37° C. was slight, and to our thought clearly incommensurate with the labor involved. Further-

more, there are substantial grounds for believing that at times the data obtained from the cultivation of water bacteria at 37° C. are misleading, owing to inability by ready means to distinguish uniformly between negative results from positive growths and negative results due to the absence of growth caused by the high temperature. Such data, even if occurring at fairly rare intervals, can lead ultimately only to serious confusion.

Period of Development.—This portion of bacterial procedures has not been standardized to the degree which seems to be imperative for those workers who have in view the object stated in the introduction of this paper. A standard period of development involves the consideration of the following factors:

1. It should not be so short as to exclude a considerable portion of the definiteness of the recorded characters of the given bacterium.

2. It should not be so long as to add unnecessarily to the tediousness of the methods and to difficulties in their applications to practical problems of medical and sanitary science; nor increase the likelihood of complications arising from contamination of the culture or the evaporation of the medium; nor force the constant use of rubber caps to protect the contents of the tubes, as it is found that such a practice affects some growths through the exclusion of oxygen.

3. It must of necessity be an arbitrary limit, lying between the wide extremes now employed by various workers, and filling in a conservative manner an intermediate position with reference to the two factors above stated.

At the outset of our present studies, it was the custom to keep all cultures for four weeks before the final descriptions and tests were made. It was learned, however, after twenty bacteria had been carefully studied, that substantially no changes of specific value were ordinarily obtained after the tenth day; and it was not until this period had elapsed that satisfactory data in several instances could be obtained upon the liquefaction of gelatine, a test which occupies a prominent place in the present bacterial methods.

Accordingly, it was decided to adopt ten days as the standard period of development in our work, and disregard in our classifications any change which by chance might be noted beyond that time, should

the cultures be preserved. It is not to be supposed that the writers are unmindful of the fact that in some instances well-marked changes may take place after the tenth day of cultivation, notably with reference to chemical changes associated with fermentation processes as in the case of milk; but it is our position that under such conditions changes of differential value are seldom obtained, and we are not yet ready to accept that they deal with species rather than with races of bacteria, in the present state of bacterial classification.

IV.—PROCEDURES DIRECTED TO FULFILL THE NECESSITY OF ARRANGING WATER BACTERIA IN GROUPS AS A PRELIMINARY STEP TOWARDS CLASSIFICATION.

Irresistibly bacteriologists working in this field have been drawn towards efforts having for their purpose the arrangement of fairly similar forms into subdivisions, which facilitate the comparative study of closely allied species or races of bacteria. Various workers have referred to their efforts in this direction as "groups," "synopses," "summaries" or "classes" of the bacteria which they studied. The importance of such steps, which has been set forth by a number of writers in the past few years, is probably conceded by substantially all workers in this line. Recently, the time-consuming task of arranging, so far as practicable, the recorded species of bacteria to date into twenty-five classes was completed by Chester* and his contribution has proven to be very helpful to us.

Many bacteriologists in the past, if not at the present time, have been inclined to move with much slowness in efforts to arrange bacteria into groups, on the ground that they could not see their way clear towards a *natural* grouping. It is of course true that with present methods of bacteriology all schemes of grouping bacteria are purely arbitrary; but it is also true that there is no probability of any one ever devising a bacterial grouping which is natural; or, in the immediate present, one which will be permanent and acceptable to all workers.

The arrangement of bacteria into groups is wholly a matter of con-

* A preliminary arrangement of the species of the Genus Bacterium by Frederick D. Chester. From Report of the Delaware College Agricultural Experiment Station, 1897.

venience, undertaken to assist in making comparative studies of similar forms and of data related thereto. While it is true that each bacteriologist might have a different method of grouping, which in his own hands was satisfactory for the purpose to which it was put, and therefore successful, yet it is obvious that a grouping used by many workers in common would lead to more systematic results and more rapid improvement of current methods.

According to our experience the present methods of grouping water bacteria are not wholly satisfactory for the following reasons:

1. The groups are too many in number, and depend for their separation upon results consequent upon immediate environment, and not upon inherent characteristics of a specific nature.

2. Following the views of biologists in other fields the importance attached to morphological data is greater than justifiable, and these data are used to the exclusion of results of more definite physiological tests.

In connection with the second of these points, it may be stated that we have had considerable difficulty in deciding uniformly as to the form (under apparently all ordinary conditions) of those very plump bacilli which at one time were specified as bacteria, and which could quite properly be classed as micrococci. Such forms are quite prevalent in some waters, although they seem to include only a very few species. With regard to spore formation and motility, we feel quite sure of our data upon these points when we make use of our regular custom of applying the heat test for spores, and for motility when we compare preparations in hanging drops of water and of formaldehyde.

The following tests are used at this laboratory to obtain data for the separation of water bacteria into groups to aid in their study and classification.

1. Fluorescence and chromogenesis.
2. Liquefaction of gelatine.
3. Well-marked characteristics of typical gelatine plates.
4. Fermentation of carbohydrates.

By means of the preliminary cultivation methods described in Section I of this paper, the preparation of media as referred to in Section II, and the standard temperature and period of development as outlined in Section III, the writers have found that from the four

tests above stated a practically constant arrangement of water bacteria may be obtained as indicated by the following outline:

TABLE VI.

DATA FOR ARRANGEMENT OF WATER BACTERIA IN GROUPS.

Water Bacteria.

- Fluorescent.
- Non-Fluorescent.
  - Chromogenic.
    - Red, Orange, Yellow, Violet.
  - Non-Chromogenic.
    - Gelatine liquefied.
      - Characteristic colonies on gelatine plates.
        - Proteus form.
        - Subtilis form.
      - Non-Characteristic colonies.
        - Fermentation of carbohydrate.
          - Gas production.
          - No gas production.
        - Non-Fermentation.
    - Gelatine not liquefied.
      - Fermentation of carbohydrate.
        - Gas production.
        - No gas production.
      - Non-Fermentation.

From the above schedule thirteen groups of water bacteria are obtained as follows:

Group I. All fluorescent forms.

Group II. All red chromogenic forms.

Group III. All orange chromogenic forms.

Group IV. All yellow chromogenic forms.

Group V. All violet chromogenic forms.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming proteus-like colonies on gelatine.

Group VII. All non-fluorescent, non-chromogenic, gelatine-liquefying bacteria, forming subtilis-like colonies on gelatine.

Group VIII. All non-fluorescent, non-chromogenic, non-proteus- and non-subtilis-like bacteria, which liquefy gelatine and ferment carbohydrate with the production of gas.

Group IX. All bacteria conforming to the specified characteristics of Group VIII, except that fermentation of carbohydrate takes place without the formation of gas.

Group X. All bacteria conforming to the specified characteristics of Group VIII, except that no fermentation of carbohydrate occurs.

Group XI. All non-fluorescent, non-chromogenic, non-gelatine-liquefying bacteria, which ferment carbohydrates with the production of gas.

Group XII. All bacteria conforming to the specified characteristics of Group XI, except that fermentation of carbohydrate takes place without the production of gas.

Group XIII. All bacteria conforming to the specified characteristics of Group XI, except that no fermentation of carbohydrate occurs.

The features upon which the above groups are based we are led to consider, arbitrarily, as "fixed characters." Being looked upon as such, it becomes necessary that certain provisions shall be observed in their consideration in order that the features true of one group may not become diffused into the one closest allied. Chromogenesis, for instance, upon which the separation of four of the above groups are based, rarely appears the same upon two different media owing mainly to differences in their composition. For obvious reasons we have

deemed it advisable to adopt a specific medium (or media) for a given observation and the conditions governing the chief features of the first five groups are therefore briefly given, as follows:

Group I.—Fluorescence should be observed exclusively in agar tube cultures; and, since a given reaction will not under all conditions be found applicable to the production of pigment for all bacteria, it is necessary that agar of three reactions should be used in this test, namely, 1.5 per cent acid, neutral, and 1.5 per cent alkaline, to phenolphthalein.

Groups II, III, IV, and *V.*—The hues or shades of color produced by the growth of certain bacteria upon culture media are well understood to be admixtures of certain colors. Most prominent among these mixed colors is that of yellow and its modifications. While the reds and violets are readily handled, it will require some study to deal effectually with the yellow and orange chromogens. We have learned that colors which are neither yellow nor orange must be set aside and placed among the non-chromogenic forms. Brown and grey-yellow colors frequently observed are instances of this character.

We have found that the most constant results in the study of chromogenic bacteria are obtained from agar tube cultures.

The colors embraced in the above four groups may be briefly explained as follows:

Red.—That color produced by B. prodigiosus on agar.

Yellow.—That color produced by the growth of Sarcina lutea on agar with the dividing line between this and the orange group at the yellow-ochre hue produced by the growth of B. ochraceus on agar.

Orange.—This color begins just below the yellow ochre. The true orange color is the same as that produced by the growth of B. aurantiacus on agar.

Violet.—The same as that color produced by the growth of B. violaceus on agar.

V.—ON THE NECESSITY OF EMPLOYING FOR PURPOSES OF CLASSIFICATION OF WATER BACTERIA DATA OF DEFINITE (POSITIVE OR NEGATIVE) INFORMATION, AND THE EXCLUSION OF THOSE DATA WHICH ARE NOT SHARPLY DEFINED TO A UNIFORM DEGREE.

Upon studying the literature of the differentiation of water bacteria, it is readily apparent to practically all bacteriologists, as was brought out in the instructive paper by Dr. Wyatt Johnston,* that the data now available upon this subject are weak and lacking in two notable ways:

1. Too many of the data, to which a differential value is given, are so indefinite that there is no assurance that the observations could be regularly duplicated even by the original worker; and, accordingly, the records of such work when published not only fail to advance the subject in a substantial manner, but even complicate and confuse matters to a serious degree.

2. There are too few data which may be called definite and which all experienced workers with good technique have reasonable expectations of confirming uniformly.

Among the principal objects of the work of the Bacteriological Committee in setting forth standard procedures was, by the use of uniform and improved methods, to increase the number of definite data by the elimination of certain ones from the first of the above classes and their transposition to the second class.

To what degree these efforts will meet with success can of course be told only after the experience of a large number of workers has been recorded, discussed and brought to a fairly agreeing consensus of opinion.

As a result of our experience of the past ten months in applying the procedures of the Committee, the writers find that in that time their views have become somewhat modified. We have become impressed with the absolute necessity of placing the subject of the classification of water bacteria upon a basis such that the systematization

* On Grouping Water Bacteria, *Journal Amer. Public Health Assn.*, Oct., 1895, p. 445.

shall have for a foundation only such data as can be readily confirmed by all capable workers employing standard methods. Or, expressed in the terms of this paper, there must be secured a foundation for future work that is made up of the results of tests which have substantially 100 for a percentage constancy, when the tests follow promptly a course of preliminary treatment to eliminate the influence of possible initial degeneration.

To some it will occur that such a decision may lead to a classification which would be very crude and fragmentary. In a measure that may be true, but to our thought the procedures of the Committee insure sufficient tests of a percentage constancy of practically 100 to lead to a fairly satisfactory set of data for primary classification, and that the advantage of all bacteriologists having a definitely located datum point in this work far outweighs at present the objection of crudeness.

The future of those tests which from our present evidence have a percentage constancy materially less than 100 is one of much uncertainty. Some of the tests will probably have their technique sufficiently improved to be elevated to the class yielding uniformly definite data. Others will probably have a field of greatest usefulness, after more or less modification in technique, in connection with the prompt identification of disease germs, and studies in the separation of species and varieties, and in tracing the life history of bacteria under different environments and from a purely scientific standpoint. To condemn them for all time seems unjustifiable at present, especially as other workers under other conditions may obtain different results.

With reference to those tests which in our hands at present seem to yield indefinite results (with a percentage constancy of much less than 100) we have no further comment to offer at this time; and list the more important ones below with the statement that from the results of our experience we are not ready to accept them as yielding data of sufficient definiteness to allow them uniformly a place at present in the classification of water bacteria. In every instance in the following list color is regarded as independent of fluorescence and chromogenesis.

LIST OF TESTS RECOMMENDED BY THE BACTERIOLOGICAL COMMITTEE WHICH HAVE BEEN FOUND TO YIELD LESS THAN 100 FOR A PERCENTAGE CONSTANCY.

MORPHOLOGY.

Capsulation. Vacuoles. Staining of spores. Presence of crystals within the cells. Pleomorphism.

BIOLOGY.

Gelatine Plate.—Size and color of colonies and growth under the mica plate.

Agar Plate.—Size, color and margin of colony.

Gelatine Tube.—Color of growth.

Agar Tube.—Extent, shape, margin and surface relief of growth.

Nutrient Broth Tube.—Time elapsing before the appearance of turbidity, deposit and surface pellicle. Color of broth. Appearance after shaking. Color, thickness and structure of pellicle. Color and amount of deposit. Quantitative reaction after a stated period.

Milk.—Color of milk. Formation of gas. Amount of whey. Quantitative reaction.

Fermentation Test. Quantitative.—Reaction of solution. Quantity of gas. Differential value of different sugars.

Lactose Litmus Agar.—Change of color.

Potato.—Color, restriction, shiny appearance and lustre of growth; and formation of gas.

VI.—THE ARRANGEMENT OF DEFINITE DIFFERENTIAL DATA IN THE FORM OF A CHART, AS A MATTER OF CONVENIENCE IN THE CLASSIFICATION OF WATER BACTERIA.

Excluding from the total number of differential tests appearing in the report of the Committee, those which were listed in the last section as insufficiently definite and constant in their value for present purposes, there remain the data from 26 tests. In the light of our experience these results can be recorded as positive or negative, with little or no material loss as to explicitness and with a decided gain as to convenience and uniformity of expression. From our studies of the

BACILLUS PYOCYANEUS AND ITS PIGMENTS.

By EDWIN O. JORDAN, Ph. D.
(*From the Bacteriological Laboratory of the University of Chicago.*)

The blue or blue-green stains that sometimes appear upon surgical dressings long ago attracted the attention of observers and, even before the cause of the phenomenon had been discovered, Fordos* carried out some important investigations upon the nature of the coloring substance. In 1882 Gessard† proved that the color is produced by a bacillus (B. pyocyaneus) which he was able to isolate in pure culture, and whose morphological and physiological characters he carefully described. Since Gessard's discovery, B. pyocyaneus has been frequently subjected to the crucible of experiment and its biological peculiarities have been scrutinized with a degree of minuteness not yet exercised in the study of most microorganisms.

Attempts to determine the nature of the pigment, and the conditions under which the chromogenic property is manifested have not, however, led to concordant results. At one extreme we find an investigator‡ who maintains that only one pigment is formed by the different "races" or "varieties," and that this pigment is identical with the ordinary blue-green fluorescent substance produced by the growth of a number of common water bacteria, and who also is of opinion that the several "races" differ chiefly in their power to produce ammonia, a substance that, like other alkalies, modifies the color of the fluorescent pigment; at the other extreme we find observers§ who claim that one and the same variety of B. pyocyaneus is able to produce as many as four distinct pigments, black, blue, green and

* *Compt. rend. Acad. des sciences*, 1860, li, p. 215.

† La pyocyanine, Thèse. Paris, 1882.

‡ Thumm, Beiträge zur Biologie der fluorescierenden Bakterien, *Arb. a. d. Bact. Inst. d. techn. Hochschule zu Karlsruhe*, 1895, i, 291.

§ Charrin and de Nittis, *Compt. rend. Soc. de biol.*, 1898, 10. s., v, 721.

yellow, at the same time and in the same culture medium; while between these extreme positions are found some investigators who hold to the existence of different races or varieties of B. pyocyaneus, each race being characterized by the ability to produce a different pigment or pigments; there are also some who maintain that by varying the nutrient substratum one and the same race may be compelled to form differently colored metabolic products.

In the existing chaotic condition of bacterial classification and description, it behooves all investigators to move circumspectly in the matter of identifying the "species" or "races" with which they are working, and in the present instance I have thought it desirable to describe with some fulness the cultures that I have employed.*

I have made use in all of seven cultures. All these have certain characteristics in common: The bacteria are small bacilli with rounded ends, and for the most part average from 0.3μ-0.5μ in breadth by 1μ-2μ in length, but there is considerable variation upon different media, and these dimensions are frequently exceeded in one and the same culture. They are sometimes grouped in short chains, but are usually single. All are actively motile. Spores have never been observed. The bacilli stain readily with the ordinary aniline dyes, but lose the stain when treated by Gram's method. Growth under the mica plate, when it occurs at all, is very scanty, and none of the cultures produces pigment in the absence of oxygen. All grow more luxuriantly at 37.5° C. than at the room temperature. Gelatin is liquefied by all. The origin of the various cultures was as follows: Four, designated respectively as "B. pyocyaneus α, Gessard," "B. pyocyaneus β, Ernst," "B. pyocyaneus pericarditidis" and "B. pyocyaneus γ, Freudenreich," were sent to me from Kral's collection; one ("B. pyocyaneus, Mich.")† was sent to me through the kindness of Dr. Novy of Ann Arbor; one ("B. pyocyaneus, Albany") was obtained at an autopsy "from a focus of broncho-pneumonia" (Jan., 1899) and was sent to me through the courtesy of Dr. George Blumer

* I am indebted to Mr. H. E. Davies and Mr. E. E. Irons for assistance in the study of these cultures.

† Dr. Novy informs me that the parent culture was brought by him from the Berlin Hygienic Institute in 1888.

of the Bender Hygienic Laboratory; and one ("B. pyocyaneus, Rush") was furnished me through the kindness of Mr. Danielson of the Rush Medical College and was originally obtained (March, 1898) from the body of a guinea-pig that had died after inoculation with a fragment of diphtheritic membrane.

These cultures, while agreeing in certain points, showed among themselves differences which, though in some respects trivial, have proved fairly constant and characteristic. All the cultures, except B. pyocyaneus, Albany, have been in my possession for at least a year. Save where otherwise indicated, I have employed the methods recommended by the Bacteriological Committee.*

As regards morphological characters, I have been able to detect few uniform or significant differences. Variations in dimensions and grouping similar in character and extent to those commonly displayed among the varieties of the colon bacillus have, however, been observed. For example, B. pyocyaneus, Albany, when grown on potato, develops larger forms and shows a greater tendency to form chains than the other varieties. B. pyocyaneus γ displays a tendency both in broth and on potato to form short chains, and takes the stain more evenly than in the other cultures. B. pyocyaneus β is, on the average, not so large as the other bacilli.

I have observed no noteworthy differences in respect to behavior toward stains. All the cultures stain somewhat irregularly when treated with Löffler's methylene-blue, a point I have not seen mentioned by other observers. None of the seven cultures hold the stain when treated by Gram's method; on this head my observations accord completely with those of Růžička.†

Gelatin.—In the growth upon gelatin plates I have been able to discover no salient and constant differences in either the macroscopic or microscopic appearances of the colonies, although differences in the rapidity of growth and of liquefaction can be noticed. Divergences in growth upon gelatin are best seen in stab-cultures. The most rapid liquefaction occurs with B. pyocyaneus α, but B. pyocya-

* *Journ. Amer. Pub. Health Assoc.*, Jan., 1898.

† *Archiv f. Hyg.*, 1899, xxxiv, p. 153.

neus β, pericarditidis, and Albany are not far behind. B. pyocyaneus, Rush, grows at first somewhat more slowly, but in about 7 days overtakes the others and in 15 days outstrips them all. B. pyocyaneus, Mich., always lags behind the five already mentioned and the rear is brought up by B. pyocyaneus γ, which liquefies the gelatin most tardily. In all the cultures except B. pyocyaneus γ the liquefaction is at first superficial, a shallow, saucer-shaped depression forming slowly and then gradually extending to the sides of the tube. The liquefied gelatin, which is more or less colored, is sharply separated by a horizontal line from the unaltered medium below; some growth takes place along the inoculation line, but at first little or no liquefaction. Later, however, considerable liquefaction occurs along the inoculation line in B. pyocyaneus, Rush, Albany and β. The growth of B. pyocyaneus γ is of the stocking-shaped variety from the start, and even after 15 days' liquefaction has not reached the walls of the test-tube. In all a pellicle is formed on the surface of the liquefied gelatin; this is most marked in the case of B. pyocyaneus, Mich.

Agar.—Upon sloped agar there are few important divergences. The features most worthy of note are shown by B. pyocyaneus, Rush; this culture forms a very thin, delicate film which rapidly covers the whole surface and has a pronounced metallic lustre. In the other cultures the surface growth, which is projecting, is of a dull yellowish-white.

Broth.—In ordinary peptonized meat broth of the standard reaction, kept at 37.5° C., B. pyocyaneus, Rush, is the first to produce a pigment. The broth is rendered turbid by all the species and a heavy flocculent sediment is formed; a surface pellicle is produced by all, but is more tenacious in the cultures of B. pyocyaneus, Mich., than in the others.

Potato.—Interesting differences appear in the growth upon potato. The growth is luxuriant, dry, projecting and of a chocolate brown color in four cases, viz.: B. pyocyaneus, Mich., γ, β, and pericarditidis. The growth produced by B. pyocyaneus α is moist and glistening, but in other respects resembles that of the four cultures already mentioned. The potato itself is not discolored by these five species.

B. pyocyaneus, Rush, however, colors the potato a beautiful deep blue and B. pyocyaneus, Albany, imparts to it a greenish hue, the color in the latter case developing less rapidly and never becoming so intense as in the former. The growth of B. pyocyaneus, Rush, is more spreading and less projecting than the others.

The "chameleon phenomenon," as it was termed by P. Ernst,* is manifested by B. pyocyaneus, Rush, but by none of the others. The chameleon phenomenon, it will be remembered, consists in the change of color observed when the growth upon potato is touched with a platinum needle. It depends doubtless on the well-known fact that a substance is produced by the growth of the bacillus which by contact with the air is oxidized to the blue pigment known as pyocyanin. The shifting play of tints seen when a colony is touched is due to the conflicting action of the atmospheric oxygen and of the reducing substances doubtless present among the metabolic products of the bacillus. The exposure of the pyocyanigenic substance to the air affords an opportunity for the development of the blue color. I may here anticipate the remainder of this paper so far as to state that my observations indicate that the reason for the appearance of the chameleon phenomenon in some races of B. pyocyaneus and not in others lies in the varying degree in which pyocyanin is produced. Those races that produce pyocyanin vigorously and abundantly manifest the "chameleon phenomenon;" others, in which the pyocyanigenic function is weaker or altogether absent, are unable to display this peculiarity.

Indol.—Only two of the cultures possess the power of forming indol in any quantity in Smith broth; these are B. pyocyaneus, Rush, and B. pyocyaneus, Mich., the former showing itself much the more vigorous in this particular. B. pyocyaneus, Albany, produced a very slight amount of indol and the other four never gave a positive reaction. An attempt was made to accentuate or develop the indol-forming power by passing the organism through a succession of peptone cultures according to the method suggested by Peckham,† but

* *Zeitschr. f. Hyg.*, ii, p. 369.

† *Journal of Experimental Medicine*, 1897, ii, p. 549.

without avail so far as the awakening of any latent indol-forming power was concerned. A few such transfers, however, exalted perceptibly the indolfacient power of B. pyocyaneus, Rush, and to a lesser degree that of B. pyocyaneus, Mich. Freshly isolated cultures of B. pyocyaneus are, in general, recorded as yielding a positive indol reaction.*

Milk.—All the cultures curdled litmus milk at 37.5° in about 2 days. B. pyocyaneus γ, however, was usually 24-36 hours behind the other cultures. The color of the litmus was discharged, but on testing the reaction of the milk at the expiration of 2 days, and again after 10 days, it was found alkaline in all cases. When the cultures were allowed to stand the casein was slowly digested. Control tubes showed no change. At the room temperature the action upon milk was similar, but less rapid than in the thermostat, curdling not beginning until after the lapse of 5 or 6 days.

Reduction of Nitrates.—In nitrate broth B. pyocyaneus, Rush, reduced nitrates to nitrites quickly and completely; B. pyocyaneus α showed a much slighter reducing power, and the other five cultures gave a negative reaction. Sewerin† observed active reduction of nitrates in a culture of B. pyocyaneus recently isolated by himself from horse-dung. Dyar‡ observed reduction of nitrates in a culture of "B. pyocyaneus" from the laboratory collection and found also that a culture of "B. fl. liquefaciens" reduced nitrates well, but not completely; while another culture found in the exudate from a sick lepidopterous larva resembled the latter in all respects except in not reducing nitrates. Thumm§ and Růžička,‖ two of the investigators who have recently carried out extended comparative studies upon this group of organisms, make no statements regarding reduction of nitrates.

Production of Gas.—None of the cultures produced gas in 2 per cent glucose broth in the fermentation tube.

* Cf. e. g. Růžička, *Arch. f. Hyg.*, 1899, xxxiv, p. 162, and Lartigau, *Journal of Experimental Medicine*, 1898, iii, p. 604.

† *Centralbl. f. Bakt.*, II. Abth., 1897, iii, p. 504.

‡ *Annals of N. Y. Acad. of Sciences*, 1895, viii, p. 322.

§ *Op. cit.*

‖ *Op. cit.*

Pigment Production.—It is in connection with the production of pigment that the most interesting, and, in some respects, the most significant differences among my seven cultures are to be noted. One culture (B. pyocyaneus, Rush) produced only one pigment, the typical, unmistakable pyocyanin; the other six, with the exception of B. pyocyaneus γ, produced both pyocyanin and fluorescent pigment: the variety labelled B. pyocyaneus γ, when it first came into my possession, produced only the fluorescent pigment, and during the year in which I have had it under observation it has lost the power of forming any pigment at all, and none of the efforts I have made to restore this power by growing the organism in media known to be favorable to the fluorescigenic property has yet proved successful.

I have made a rather detailed study of the conditions under which the pigments are produced.

Fluorescent Pigment.—The general conditions that permit the formation of the blue-green fluorescent pigment are exactly similar to those that I have elsewhere described* as necessary to the manifestation of this property by other bacteria of the same group. The presence of both phosphate and sulfate is essential; the associated cation is a matter of indifference—sodium, potassium or magnesium being equally available. Asparagin, ammonium succinate, ammonium lactate and ammonium citrate all proved suitable for the development of the fluorescent pigment.† In ammonium urate solution (amm. urate, 0.05 per cent) a small amount of pigment was produced by the cultures designated as B. pyocyaneus α and B. pyocyaneus, Mich., but the others gave only the merest trace of color. In ammonium acetate solution B. pyocyaneus, Albany, B. pyocyaneus, Mich., B. pyocyaneus α, B. pyocyaneus β and B. pyocyaneus pericard. produced pigment abundantly. Solutions of ammonium tartrate, oxalate and formate proved entirely unfavorable for the production of the fluorescent pigment, none of the species employed being able to form any pigment whatsoever in media con-

* *Botanical Gazette*, xxvii, Jan., 1899, p. 19.

† The standard solution used contained in each case 0.5 per cent of the ammonium salt and 0.1 per cent each of neutral sodium phosphate and magnesium sulfate.

structed from these salts. In the ammonium tartrate solution, abundant growth occurred in the culture of B. pyocyaneus pericard. and B. pyocyaneus, Albany, and a somewhat less abundant growth in the culture of B. pyocyaneus, Mich. In no case, however, was any pigment formed. To put the whole matter concisely, *the study of five varieties of B. pyocyaneus which produce fluorescent pigment in suitable media has entirely confirmed the conclusions arrived at in my previous work upon the production of fluorescent pigment by bacteria.**

Pyocyanin.—In spite of the inability of Thumm† during his extended study of pigment production to discover any evidence for the existence of pyocyanin, there is no doubt whatever that a blue pigment with definite optical and chemical characters is formed by some bacteria belonging to the same group of organisms that Thumm studied. The cause of Thumm's failure will be made clear presently. All the cultures that I have worked with, excepting the undoubtedly degenerate B. pyocyaneus γ, have formed pyocyanin, although in varying degrees and under different conditions. The blue pigment termed pyocyanin can be readily distinguished from the blue-green fluorescent pigment. It is soluble in chloroform while the fluorescent pigment is not; viewed by artificial light, pyocyanin is as brilliant and distinct as by daylight, while the fluorescent pigment loses its vivid emerald green tint altogether and appears a dim muddy yellow. Either of these tests for the detection of pyocyanin in the presence of the fluorescent pigment can be applied very simply. A small amount of chloroform shaken up with a fluid culture becomes, within a few seconds, a deep blue if pyocyanin be present. If cultures when viewed by daylight appear to contain nothing but the fluorescent pigment—as under certain conditions was the case with B. pyocyaneus α and B. pyocyaneus, Albany—pyocyanin can be easily detected by viewing the cultures under gaslight, when any pyocyanin present will be plainly seen owing to the blotting out of the fluorescent substance that in daylight effectually screens the other pigment.

* *Botanical Gazette*, Jan., 1899.

† *Op. cit.*, p. 367.

Another well-known difference between the pigments may also be mentioned here. Pyocyanin turns red when treated with acid, while the fluorescent substance becomes colorless, both pigments regaining their original hue when the solution is again rendered alkaline. In all the cultures that I have worked with, pyocyanin is produced more slowly than the fluorescent pigment. B. pyocyaneus, Rush, develops pyocyanin at the room temperature only after three to four days' growth, while the races that produce the fluorescent pigment at all vigorously show color 24-48 hours earlier.

One of the races that I have used for experiment, B. pyocyaneus, Rush, does not produce the fluorescent pigment, and for this reason has been particularly serviceable for the study of the conditions under which pyocyanin is produced. In ammonium succinate or asparagin solutions, prepared according to the formulæ given above, pyocyanin is produced abundantly, and I have found that after ten transfers in ammonium succinate, made at intervals of 6-8 days, the power of this race to form pyocyanin persists unimpaired, and at the end is manifested as energetically as at the beginning. This race also produces pyocyanin in lactate, acetate, and citrate solutions. A very faint blue tinge, due to pyocyanin, appears in the urate, but in the solutions of tartrate, oxalate and formate no pigment at all is formed, although a very good growth occurs in both tartrate and oxalate media. Pyocyanin is also formed by this race in beef broth and upon ordinary nutrient gelatin and agar.

Upon testing the behavior of the other varieties in the several media interesting differences came to light. B. pyocyaneus, Mich., and B. pyocyaneus, Albany, produced both pyocyanin and fluorescent pigment in succinate, lactate, acetate and citrate solutions, while B. pyocyaneus α, B. pyocyaneus β and B. pyocyaneus pericard. produced only the fluorescent pigment in these media, and did not develop any pyocyanin even after 30-40 days' growth. In the urate solution a small amount of fluorescent pigment, but no pyocyanin, was formed by B. pyocyaneus, Mich., and B. pyocyaneus α; B. pyocyaneus β did not form any pigment although rendering the solution turbid. Neither pyocyanin nor fluorescent pigment was formed by these four races in tartrate, citrate or formate media.

In standard beef broth B. pyocyaneus, Mich., and B. pyocyaneus, Albany, produced pyocyanin scantily, and the other three races formed only a small amount of fluorescent pigment. In broth from which the muscle sugar has been removed by Smith's method of inoculation with B. coli the fluorescent pigment is produced much more abundantly, but I have not observed so great a difference in the formation of pyocyanin. B. pyocyaneus, Rush, produces some pyocyanin in broth to which 2 per cent of glucose has been added, but the pigment appears more slowly than in Smith broth and the culture assumes a yellow tinge. B. pyocyaneus, Mich., also produced some pyocyanin in a solution composed of 0.2 per cent asparagin; 0.2 per cent glucose; and 1 per cent potassium sulfate. Nicolle and Zia Bey* note that their cultures produced the blue pigment in "milieux sucrés."

In nutrient gelatin all five cultures produced both pyocyanin and the fluorescent pigment; this is the only medium I have yet found in which B. pyocyaneus α, B. pyocyaneus β, and B. pyocyaneus pericard. form pyocyanin.

Further experiments have been made with the three races capable of forming pyocyanin in non-proteid media with a view of determining the chemical elements essential to this activity.† The conditions are distinctly different from those governing the production of the fluorescent substance. Neither sulfate nor phosphate is essential. In a nutrient solution composed of asparagin, 0.2 per cent; magnesium sulfate, 0.1 per cent; sodium chloride, 0.5 per cent, B. pyocyaneus, Rush, produced pigment almost as abundantly and rapidly as in a standard sulfate-phosphate solution, and the pyocyanigenic power was not perceptibly weakened during four transfers in the same medium. The same thing proved true of B. pyocyaneus, Mich. If only one salt, as sulfate alone or phosphate alone, was used with the asparagin some pyocyanin was formed, but the amount in such cases was much less than under conditions more favorable to the multi-

* *Ann. de l'Inst. Pasteur*, 1896, x, p. 670.

† The cultures were kept at the room temperature unless otherwise indicated.

plication of the organism, although even in a pure 1 per cent asparagin solution some pyocyanin is formed by the three races named. It is entirely clear, therefore, that the pyocyanigenic property is in a sense more intimately bound up with the life processes of these organisms than is the fluoresceigenic, since it is less dependent upon the presence of definite chemical compounds in the culture medium. Gessard* has also noticed the production of pyocyanin in a medium to which no phosphate was added, but is inclined to attribute it to the presence of small traces of phosphate carried over in the first sowing. This is of course possible, but the amount of phosphate in such a solution must be exceedingly small and the pyocyanigenic power of the organism certainly shows no such abject dependence upon the presence of this salt as is manifested in the case of the fluorescent pigment. In a medium containing 0.2 per cent asparagin, 0.1 per cent magnesium sulfate, and 0.001 per cent of neutral sodium phosphate B. pyocyaneus, Mich., produces both pyocyanin and fluorescent pigment. If the phosphate be altogether omitted pyocyanin alone is formed, and continues to be formed for at least four generations of successive transfers, which is as far as I have followed it in this species. The medium is prepared from recrystallized C. P. salts as free from phosphate as possible, and does not respond to the delicate phosphomolybdate test. Thumm† refers to Gessard's statement regarding the appearance of a blue color in the absence of phosphate and asserts that he has observed the same phenomenon in cultures of all the fluorescent bacteria that he has studied and explains the effect in this way: "Diese Färbungen sind jedoch nie auf Pyocyaninbildung sondern einfach auf Lichtbrechungserscheinungen zurückzuführen, indem jede leicht getrübte Flüssigkeit einen blauen Schimmer zeigt." Such an explanation in respect to the effects that I have observed is totally inadmissible, since I have demonstrated the presence of pyocyanin by all the applicable tests. Thumm's failure to obtain any pyocyanin here and in his other experiments with non-proteid media undoubtedly lay in the fact that the races with which he worked, whatever might have

* *Ann. de l'Inst. Pasteur*, 1892, vi, p. 810.
† *Op. cit.*, p. 367.

been their earlier history, at the time of his investigation were unable to produce pyocyanin in media constructed from ammonium salts. Three of the races in my possession show this same incapacity and the fact should be noted that they, like the races employed by Thumm, had been for some time under artificial cultivation and were perhaps obtained from the same laboratory. One of Thumm's* races seems to have behaved exactly like my own culture of "B. pyocyaneus γ" in losing its power to produce even the fluorescent pigment after it came into his possession.

Gessard† is inclined to lay great stress upon the production of pyocyanin in very simple media. "La fonction essentielle du bacille pyocyanique est donc la fonction pyocyanogène. . . . . C'est à bon droit qu'elle entre dans son nom spécifique. C'est celle dont ne le séparent pas même ces conditions précaires d'existence, cette véritable misère physiologique qui aboutit à la mort, après un petit nombre de générations dans le milieu dépourvu de phosphate. L'autre fonction est surajoutée; elle n'est pas essentielle." I see no reason for establishing so sharp a distinction between the pyocyanigenic and the fluorescigenic function. It can hardly be doubted that cultures like B. pyocyaneus α and B. pyocyaneus β, which at present produce fluorescent pigment but no pyocyanin in ammonium succinate solutions, are merely modified descendants of races which when first isolated were more vigorous producers of pyocyanin. In fact there can be no objection to the view that the cultures of "B. pyocyaneus" that are not uncommonly found in laboratory collections and that secrete only the fluorescent pigment are nothing but degenerate offspring, so to speak, of a parent stock that originally produced both pigments. Such evidence as we possess is distinctly in favor of the view that the pyocyanigenic property is sometimes spontaneously lost while the fluorescigenic persists. The former "function" can hardly be looked upon, then, as more "essential" than the latter.

Cultural Modifications.—Gessard‡ was among the first to call atten-

* *Op. cit.*, p. 341.
† *Ann. de l'Inst. Pasteur*, vi, pp. 811, 812.
‡ *Ann. de l'Inst. Pasteur*, 1891, v, p. 65.

tion to the possibility of manufacturing new races of B. pyocyaneus by subjecting cultures to different conditions. According to this author the "typical" race, yielding both pyocyanin and fluorescent pigment, could be converted into a race possessed only of the fluorescigenic property either by the action of heat (five minutes' exposure to 57° C.) or by passage through the body of a rabbit; a pyocyanigenic race, on the other hand, could be created by growth of the typical race for a series of generations upon egg-albumen; while by the action of heat, by passage through the body of a rabbit or by spontaneous degeneration the pyocyanigenic race could be changed into a race incapable of producing any pigment; by the action of heat, too, the fluorescigenic race could be converted into a non-pigmented variety.

Acting upon these statements I have attempted to abolish or modify the pyocyanigenic power of B. pyocyaneus, Rush, by several methods. One of the first to be carried out was the passage through successive cultures in media particularly favorable to the production of fluorescent pigment, with the aim of developing some latent fluorescigenic power, but, as I have stated above, a series of ten generations in a standard succinate-sulfate-phosphate solution did not perceptibly affect the amount of pyocyanin produced and there was not the slightest approximation towards a fluorescigenic variety; on the other hand, B. pyocyaneus α, when grown in the same sulfate-phosphate medium, continued to produce only the fluorescent pigment after a parallel series of transfers. In ammonium tartrate solution no pigment is produced by either variety during the course of five generations, but on transfer to ammonium succinate the pigment characteristic of each reappears in the first generation.

Following the method suggested by Gessard, I subjected a fluid culture of B. pyocyaneus, Rush, to a temperature of 57° C. for 5 minutes, but this procedure failed to destroy the pyocyanigenic power, although development was slightly retarded in the subculture. Nicolle and Zia Bey* obtained a result similar to my own when they applied the heat method to some recently isolated cultures of

* *Ann. de l'Inst. Pasteur*, x, p. 669.

B. pyocyaneus, but Jakowski* has corroborated Gessard's statement, that the pyocyanigenic power is abolished in a culture warmed for 5 minutes at 57° C. Different races possibly differ in this regard. Neither Jakowski nor Nicolle and Zia Bey on the other hand observed any loss of pyocyanigenic power when the organism was passed through the body of rabbits. My own experiments with animals were made with mice and guinea-pigs, and are hence not strictly comparable with Gessard's, but in these animals at least no alteration of the chromogenic function could be observed in cultures isolated after death from the heart's blood, the kidneys, spleen or liver. Kukula, as quoted by Růžička,† observed sometimes exaltation, sometimes attenuation of the chromogenic power when B. pyocyaneus was passed through the animal body.

A particular interest attaches to modification experiments in view of Růžička's recent comparative studies‡ upon cultures of "B. pyocyaneus" and "B. fluorescens liquefaciens." The close relationship existing between cultures of organisms bearing these names has long been recognized. Intermediate forms are common, and no hard and fast line can be drawn between the "varieties" of the two "species." The ability to grow and produce pigment at 37° C. is perhaps as distinctive of B. pyocyaneus as any single physiological character, but the well-known ability of bacteria to adapt themselves to varying temperatures and the actual existence of a whole series of "liquefying fluorescent bacteria" that vary in behavior to temperature under natural conditions do not permit us to lay much stress upon this point of difference. Růžička attaches some importance to a freer growth of B. pyocyaneus beneath the surface of gelatin or agar media, but as I have stated elsewhere in this paper, I have not found a uniform behavior in this regard among the various pyocyanin-producing cultures in my possession. As respects indol-formation, nitrate reduction, action upon milk and growth in the ordinary nutrient media, I share Růžička's opinion that almost all characters and combinations of characters can be found among members of this group of organ-

* *Zeitschr. f. Hyg.*, xv, p. 474.
† *Arch. f. Hyg.*, 1899, xxxiv, p. 173.
‡ *Op. cit.*, p. 149.

isms. One culture will form indol, grow at 37° C. and reduce nitrate; another will not form indol but will agree with the first in all other characters, and so on through a long series in which all possible combinations of physiological capacities seem to be represented among the cells of one or another culture.

Růžička does not, however, appear to grasp the full significance of the difference between "B. pyocyaneus" and "B. fluorescens liquefaciens" in the matter of pigment production. Gessard's experiments consisted simply in removing the power to form one or the other pigment from an organism originally capable of forming both pigments. I have not been able to find in the literature a single satisfactory instance of the acquisition of pyocyanigenic power by an organism primarily devoid of this property. Kruse and Pasquale* mention the discovery of an organism resembling B. pyocyaneus except in ability to produce pigment, but do not affirm that it acquired chromogenic power on cultivation. Remlinger† records the occurrence of a non-chromogenic variety in splenic pulp and states that color appeared after the sixth or seventh sowing in broth, but I have not been able to satisfy myself that this statement necessarily implies acquisition of pyocyanigenic power. Růžička‡ describes an experiment upon the transformation of one variety into another in the course of which he noticed the formation of a "gesättigt blaugrünen Farbstoff" in a typical culture of B. fluorescens liquefaciens, but his description is not sufficiently detailed to carry conviction upon the point at issue.

I have made a number of experiments designed to augment the pyocyanigenic power of my cultures and to evoke the power in cultures in which it was latent, but thus far with only indifferent success. The pyocyanigenic power of B. pyocyaneus, Rush, was very slightly but perceptibly increased by passage through a series of cultures in ordinary peptone broth and the pigment was produced more rapidly and abundantly at the end of eight transfers than at the beginning. Similar attempts to increase the amount of pyocyanin formed by B.

* *Zeitschr. f. Hyg.*, xvi, p. 63.
† *Arch. de. méd. exp.*, 1898, x, p. 167.
‡ *Op. cit.*, p. 173.

pyocyaneus, Albany, and B. pyocyaneus, Mich., did not prove successful, and no pyocyanin at all was produced by B. pyocyaneus pericard. during the course of fifteen generations. A series of sowings in nutrient gelatin did not exalt in any degree the pyocyanigenic power in the three organisms that manifested it only in this medium. In this respect there is an agreement with what is noticed concerning the indolfacient power of the organisms.

There is, as might be expected, a difference in chromogenic power among the cells of one and the same culture. B. pyocyaneus, Mich., for example, was plated from a pure culture obtained from a single colony in the usual way. From nine separate colonies selected at random from the gelatin plate nine separate tubes of standard ammonium succinate solution were inoculated. Fluorescent pigment made its appearance in one tube on the fourth day after inoculation, in five more on the fifth day, in two more not until the eleventh day and in the ninth tube no pigment appeared until the fifteenth day. After twenty-four days the cultures were tested for pyocyanin and eight tubes showed the presence of this pigment accompanying the fluorescent. The ninth tube (one of those in which fluorescent pigment appeared on the fifth day) contained no pyocyanin, and further experiment showed that the descendants of this cell were devoid of pyocyanigenic power.

Other Pigments.—The production of other pigments besides the blue and the blue-green has been mentioned by some authors. Charrin and de Nittis* observed the simultaneous formation of black, blue, green and yellow pigments in one and the same culture. The yellow or yellow-brown pigment is, as I have shown elsewhere, an oxidation product of the green fluorescent pigment and is commonly seen in old cultures and among races beginning to lose fluorescigenic power. The black pigment has been recently attributed by Gessard† to the oxidation of tyrosin. I have observed the formation of the black pigment in old agar and gelatin cultures of B. pyocyaneus, Rush, B. pyocyaneus, Mich., and B. pyocyaneus, Albany; in other words, in the races that produce pyocyanin most vigorously. B. pyocyaneus,

* *Compt. rend. Soc. de biol.*, 1898, p. 721.
† *Compt. rend. Soc. de biol.*, 1898, p. 1033.

Rush, which is by far the best producer of pyocyanin that has come into my hands, develops the black pigment most profusely. To test the relation of this pigment to tyrosin, I inoculated a solution of 0.05 per cent tyrosin (Merck), 0.1 per cent neutral sodium phosphate and 0.1 per cent magnesium sulfate with the several cultures. Growth occurred with all, but not quite so abundantly as in the ammonium succinate medium. B. pyocyaneus, Rush, formed pyocyanin quite abundantly but rather slowly, the first appearance of the pigment being noticed in 12 days; B. pyocyaneus, Mich., and B. pyocyaneus, Albany, formed both pyocyanin and fluorescent pigment, but in scanty amounts. The chromogenic behavior of these organisms in a tyrosin medium does not, therefore, differ materially from their behavior in an ammonium succinate solution. The culture of B. pyocyaneus, Rush, in tyrosin, *which was kept in the dark*, contained only pyocyanin and had no trace of black pigment at the end of 113 days. The black pigment is, in fact, due to the oxidation of the pyocyanin. Exposure of a pyocyanin solution (an old ammonium succinate culture of B. pyocyaneus, Rush) to the action of strong north light causes a change, first to a bottle-green hue, then to a brownish-black; the same change goes on in the dark in some media, but very much more slowly than in tubes exposed to daylight. This change is one of oxidation, as is shown by the fact that a dilute solution of potassium permanganate added gradually to a pyocyanin solution produces exactly the same conversion of the blue into the bottle-green and then into the black pigment, as is wrought more slowly by the action of light and air.* I have elsewhere shown† that the conversion of the fluorescent pigment into the yellow-brown depends upon a similar oxidizing process. The formation of four pigments, then, can be easily explained on the basis of the oxidation of the blue and the fluorescent pigments into black and yellow respectively. The almost uniform occurrence of a yellow-brown tint in old stock cultures of B. pyocyaneus that have lost their ability to form either pyocyanin or fluorescent pigment may be most plausibly referred, perhaps, to an accentuation of oxi-

* Charrin and de Nittis, *loc. cit.*, state that the black pigment is produced at the surface of the agar.

† *Botanical Gazette*, 1899, xxvii, p. 19.

dizing processes, and the real explanation of the loss of pyocyanigenic and fluorescigenic power may perhaps lie in the impetus given to metabolic processes of oxidation by the conditions of life in our artificial peptone media. It must not be forgotten that both pyocyanin and the fluorescent pigment are themselves oxidation products of colorless substances.

Natural Varieties.—The confusion into which the study of B. pyocyaneus and its varieties has fallen is in part due to the rapid degeneration of many stock cultures under conditions of artificial cultivation and the consequent disappearance of all chromogenic power, and in part also to the occurrence of natural varieties characterized by considerable physiological divergence. The most common natural variety, to judge from an examination of the literature, as well as from the study of a number of freshly isolated cultures that I have obtained from different sources, is the one that produces both pyocyanin and the fluorescent pigment. This is the variety that was originally described and studied by Gessard, and I would suggest that the name B. pyocyaneus, var. α be given to freshly isolated cultures possessed of this double chromogenic power. The culture with which Ernst worked* and to which he gave the designation of "α," was, so far as I can determine from his description, a culture wholly devoid of pyocyanigenic power and one that produced, while in his hands, only the fluorescent pigment.† The most natural inference is that in respect to pyocyanin production this culture was degenerate. Many cultures seem overtaken by this fate. It is perhaps fair to assume that the large number of cultures found in laboratory collections bearing the name of B. pyocyaneus, but without any power to produce pyocyanin, are degraded scions of a more vigorous parent stock.‡ It is worthy of note that the pyocyanigenic property is apparently the first to be lost. I have never observed a case where the fluorescigenic

* *Zeitschr. f. Hyg.*, ii, p. 369.

† Cf. e. g. the statement on p. 373, *op. cit.*, that the color of cultures of the α-bacillus disappears when viewed by gaslight.

‡ The failure to preserve unimpaired the original pyocyanigenic power is clearly shown in the usual inability of old cultures to manifest the "chameleon phenomenon," while most freshly isolated cultures display this power (cf., for example, Schürmayer (cited below) and Lartigau (*loc. cit.*).

power was lost by spontaneous degeneration while the pyocyanigenic persisted, nor have I found mention of such a case in the literature.

It is a matter of common experience that there is great diversity among cultures of B. pyocyaneus as regards rapidity of degeneration. Some lose the power to produce either pyocyanin or fluorescent pigment within a short time after isolation; others weaken quickly in pyocyanigenic power, but retain the fluorescigenic much longer; others again, but these are the rarer cases, show almost undiminished capacity for the formation of pyocyanin and fluorescent pigment through a long series of generations. B. pyocyaneus, Mich., is the most notable instance of the last group with which I am acquainted and is a marvelously stable variety; it has shown no attenuation in chromogenic power during the three years and a half I have had it under observation.

In the relative proportion of pyocyanin and fluorescent pigment there is likewise considerable variation among freshly isolated cultures. New varieties have even been founded by some authors upon the differing nuances of color due to varying admixtures of the two pigments, but the advisability of such distinctions is questionable. It must be acknowledged that there are found races of the variety that I have termed var. α, some of which are more or less sharply distinguished from one another by intensity and persistency of pigment production as well as by such physiological traits as the formation of indol, reduction of nitrates and growth on potato, but it must be left to future bacteriological investigation to interpret the significance of these deviations and to determine to what extent subvarieties and races shall be established.

The culture I have designated as B. pyocyaneus, Rush, produces only pyocyanin under ordinary conditions of cultivation, and has resisted all my attempts to communicate to it any fluorescigenic power. I would suggest that the name B. pyocyaneus, var. β, be employed to distinguish this non-fluorescigenic variety. I am unable to determine whether the variety studied by Ernst,* and designated by him as the "β variety," produced only pyocyanin, but the coloring of his

* *Zeitschr. f. Hyg.*, ii, p. 369.

figures (*op. cit.*, p. 372), and the peculiar liquefaction of gelatin there shown, together with the statement regarding tardy appearance of the color, suggest that this may have been the case. Schürmayer* and Jakowski† both worked with cultures of the pyocyanigenic-fluorescigenic variety, which I have called the "α" variety. Nicolle and Zia Bey‡ also appear to have been dealing with the α-variety, although they state that their four cultures were "much more pyocyanigenic than the type." I have not been able, in fact, to find in the literature any unimpeachable statement of the existence of a purely pyocyanigenic variety such as I have worked with, although it is not difficult to discover instances where the pyocyanigenic power of a culture has been recognized to be much higher than is usually the case.

The occurrence of a fluoresceigenic variety seems to be more common. The variety I have termed the α-variety frequently degenerates spontaneously into this. The chief difference between this variety and the organism called B. fluorescens liquefaciens seems to lie in the difference of temperature optimum and perhaps in behavior in the animal body (Růžička). I have not been able to confirm Růžička's observations respecting a constant difference in gelatin stab-cultures. It is a tempting hypothesis that B. fluorescens liquefaciens is a degenerate or modified form of B. pyocyaneus, but more convincing evidence than we now possess is needed to establish this point.

The natural occurrence of a non-chromogenic variety has been affirmed by Remlinger§ and others. Kruse and Pasquale‖ have recorded the finding of a non-chromogenic variety exactly like the ordinary B. pyocyaneus, but without power to produce pigment. I have not been able to obtain a natural variety of this kind. It will be generally recognized that the method of repeated transfer of such a variety from one tube of fluid culture to another (Remlinger) is open to objection unless frequent control platings are carried out. My own attempts to restore chromogenic power to spontaneously or artificially etiolated cultures have failed.

* *Zeitschr. f. Hyg.*, xx, p. 281.
† *Zeitschr. f. Hyg.*, xv, p. 474.
‡ *Ann. de l'Inst. Pasteur*, p. 669.
§ *Op. cit.*
‖ *Op. cit.*

CONCLUSIONS.

The principal conclusions that seem to me justified are as follows:

1. The fluorescent pigment formed by some varieties of B. pyocyaneus is produced under conditions identical with those governing the production of the pigment by other "fluorescent bacteria."*

2. The production of pyocyanin is not dependent upon the presence of either phosphate or sulfate in the culture medium. It is formed in non-proteid as well as in proteid media, but is not a necessary accompaniment of the metabolic activities of the organism (*e. g.* tartrate solution).

3. The power of producing pyocyanin under conditions of artificial cultivation is lost sooner than the fluorescigenic power.

4. There are greater natural and acquired differences in pyocyanigenic power than in fluorescigenic.

5. The fluorescent pigment may be oxidized slowly by the action of light and air as well as by reagents into a yellow pigment, and pyocyanin may be similarly oxidized into a black pigment.

6. A convenient separation of B. pyocyaneus into four varieties would be the following: var. α, pyocyanigenic and fluorescigenic (most common); var. β, pyocyanigenic only (rare); var. γ, fluorescigenic only (not uncommon, closely related to "B. fluorescens liquefaciens"); var. δ, non-chromogenic.

7. Except for the occasional loss of one or another function the different varieties are not so plastic as sometimes assumed, and cannot be readily converted into one another by subjection to varying conditions of life.

8. The signification and correlation of the almost countless physiological variations among the members of this group in respect to growth in gelatin, behavior to temperature, indol production, etc., remain to be determined. It is not yet clear that the variations in chromogenic power can be in any way correlated with the presence or absence of other physiological functions.

* Cf. *Botanical Gazette*, xxvii, p. 19.

A PRELIMINARY NOTE ON THE FRACTIONAL PRECIPITATION OF THE GLOBULIN AND ALBUMIN OF NORMAL HORSE'S SERUM AND DIPHTHERIC ANTITOXIC SERUM, AND THE ANTITOXIC STRENGTH OF THE PRECIPITATES.

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It has been shown by investigators that the globulin of tetanus and diphtheria antitoxic sera, when separated from the sera, carries with it the antitoxic power of the sera. It has not been proved, however, that the "antitoxin" is a globulin. My work of the past two years has been directed to the study of the proteids of different antitoxic sera, especially diphtheria antitoxic serum.

An article in the *Zeitschrift für Hygiene und Infectionskrankheiten* (1889, xxxi, p. 513) by Dr. W. Seng, entitled "Ueber die qualitativen und quantitativen Verhältnisse der Eiweisskörper im Diphtherieheilserum," suggests to me that it would be well to publish a preliminary note on my work.

When the globulin, which has been separated from horse's serum by means of magnesium sulphate and purified by dissolving in water, reprecipitating and washing with a saturated magnesium sulphate solution, is dissolved in water, and this solution of purified globulin is saturated with sodium chloride, a precipitate is formed. This I will call the room temperature precipitate (R° ppc.). If, after the removal of the R° precipitate by filtration, the temperature of the filtrate be raised to 40° C., a little more salt having been added to insure saturation, a turbidity appears in the fluid and when the temperature of 44° or 45° C. is reached the precipitation is complete for this temperature.

Proceeding in the same manner one obtains a third turbidity at 49°

C. and complete precipitation at 53° C., a fourth turbidity at 57° C. and complete precipitation at 62° C., and finally a fifth turbidity at 67° C. and complete precipitation at 72° C. These precipitates dissolve in water, except a very small quantity of that obtained at 72° C., which is insoluble in water but redissolves immediately when treated with weak sodium hydroxide.

Exactly the same reactions occur when diphtheric antitoxic serum is employed instead of normal horse's serum. In both sera the final filtrates fail to give the biuret reaction, and on boiling and subsequent addition of a little acetic acid show no turbidity. Each one of the separate fractions of the antitoxic globulin possesses an antitoxic power while the final filtrate is free from antitoxin.

After the globulin precipitated by the magnesium sulphate in both the normal and diphtheric antitoxic sera has been filtered off, the albuminous filtrate is saturated with sodium chloride. One then finds that an albuminous precipitate is not formed at room temperature. A double salt which is formed by the magnesium sulphate and sodium chloride is removed by filtration and the filtrate is raised in temperature to 56° C. when a turbidity is formed. At 61° C. the precipitation is complete. At 68° C. a second turbidity appears and can be filtered off at 72° C. At 73° C. a third turbidity appears and at 76° C. this precipitation is complete. Finally at 77° C. a slight turbidity appears and at 81° C. the precipitation is complete. The final filtrates from the 81° precipitation fail to give the biuret reaction, and on boiling and subsequent addition of acetic acid show no turbidity.

The albumin precipitated at 56° C. is soluble in water. The other three precipitates are partially soluble in water, and completely so in sodium hydroxide.

In a paper which will shortly appear I shall give the quantitative relations between the corresponding fractions of the normal and antitoxic globulin and albumin, and the antitoxic value of the fractions of the antitoxic globulin.

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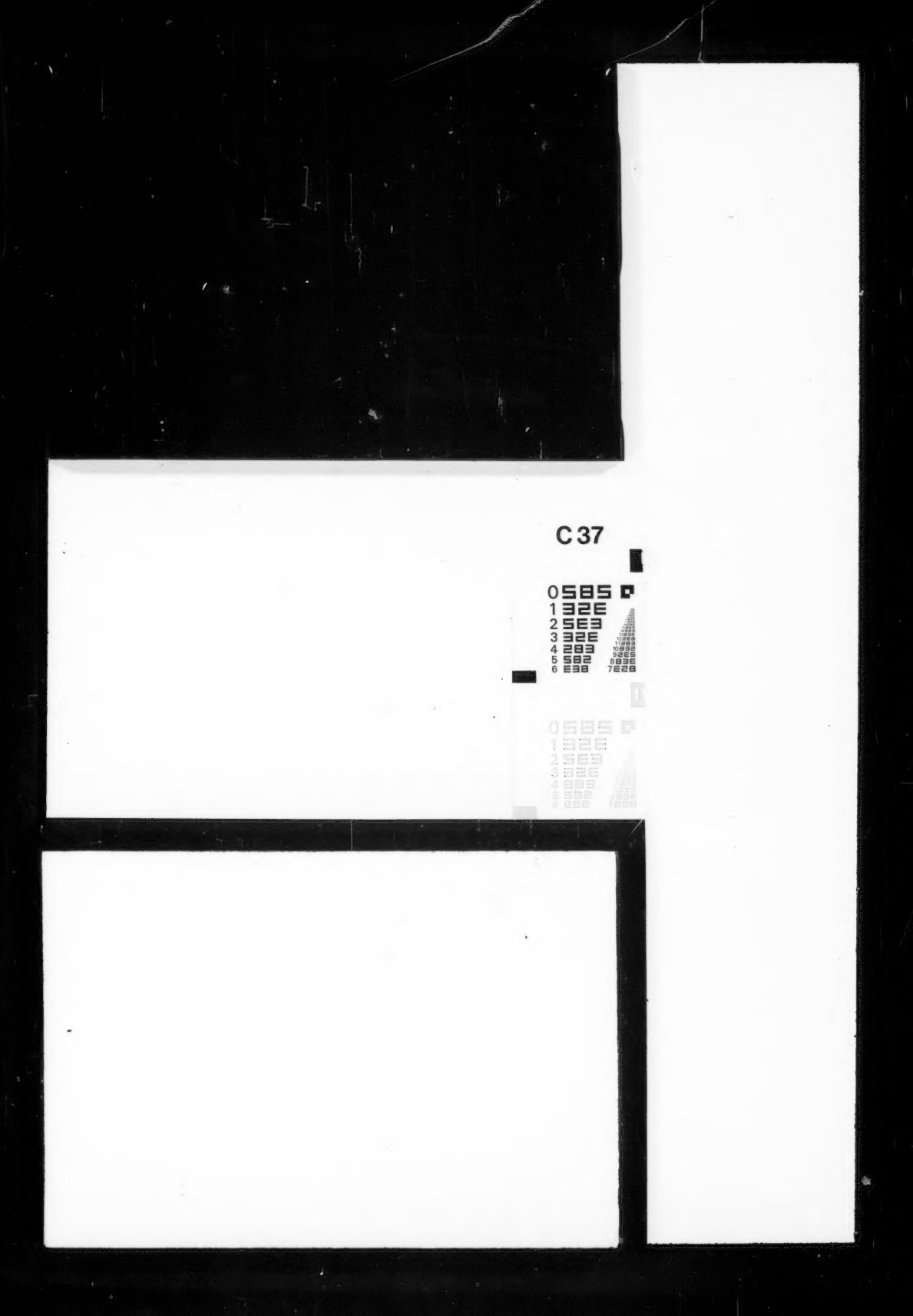
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